# The specificity of the host's immunological response to invasive nematode parasites of rats

by

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Declaration

The experiments reported in this thesis were done by myself. The thesis was composed by myself and has not been submitted in any previous application for a degree.

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## 1. Abstract

The amount of inter- and intra-specific cross-immunity between three nematode parasites was studied using *S.venezuelensis* and two strains of *S.ratti*. Reciprocal cross-challenge experiments were carried out where rats were primed with either full infections or transferred immune mesenteric lymph node cells. The response of mesenteric lymph node cells obtained from uninfected donors, or donors infected with 2000 third-stage larvae of the relevant parasite, to *in vitro* stimulation with homologous and heterologous parasite antigens was also studied.

Results of experiments using full infections demonstrated that prior infection with as few as 91 third-stage *S.ratti* larvae produced up to a 90% reduction in the worm burden on day 8 and 80% reduction in the number of eggs *in utero* per worm after homologous challenge, regardless of the strain used. Reciprocal cross-challenge experiments using the two *S.ratti* parasites revealed a difference in the level of protection produced post-challenge, which depended on the strain used at priming. Immunising rats with the heterogonic strain produced a significantly greater resistance against homologous compared with heterologous challenge. If the homogonic strain was the priming agent there was no significant difference in the relative protection produced by either type of challenge.

Adoptive transfer studies revealed a strain-specific, dose-dependent effect in homologous challenged rats, primed with 1-2 x $10^8$ immune mesenteric lymph node cells from donors infected with either an "exact" dose of less than 100, or 2000, third-stage larvae. If the homogonic strain of *S.ratti* was used to prime the cell donors then a significantly greater immunity was expressed by

recipients of cells from donors given the higher infection dose. However, if the heterogonic strain was used to prime cell donors there was no significant difference in the level of resistance transferred by the two cell populations. Reciprocal cross-immunity studies using this technique confirmed that the two *S.ratti* strains expressed different functional antigens and that asymmetry found from full infections was based in "immunological" events. They also showed that all three *Strongyloides spp.* parasites possessed common functional antigens.

It was found that primary infection rates with *S.venezuelensis* in rats of the Edinburgh Wistar colony were low, so that direct quantitative comparison between *S.ratti* and *S.venezuelensis* was not possible in all contexts. Various methods were attempted to overcome this problem, i.e. priming rats with surgically transferred worms, trickle infections, heat-killed third-stage larvae, and full infections using immature rats. Of these, some success was achieved with the last named in confirming that all three parasites expressed common antigens and that there was a difference between the two strains of *S.ratti*. *S.venezuelensis* appears to be antigenically more similar to the heterogonic strain of *S.ratti* than to the homogonic variety of that species.

Theories to explain how the asymmetery in the host's immune response against the two *S.ratti* strains might arise are proposed, and the significance of these results in the general framework of experimental immunology of nematode infection is discussed.

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# CHAPTER 1

## GENERAL INTRODUCTION

From the 1930's to the early 1970's, experimental immunology of nematode parasitism of warm blooded animals was dominated by attempts to ascertain whether humoral rather than cellular components of the immune system were the more important in parasite expulsion. The idea fundamental to studies over this period was that the two pathways could act independently, even though their effects were additive. A breakthrough came in 1971, when Ogilvie and Jones first clearly defined the idea that worm rejection was due to a "two step" process, specific models of which had already been suggested (Barth *et al*, 1966). According to this general scheme each step is only effective in synergy with its partner, a concept which revitalised research in this field. Ogilvie and Jones applied the principle to studies of *Nippostrongylus brasiliensis* in rats and, consistent with the knowledge at that time, proposed a first step in which specific humoral antibody inflicted damage on the worms but did not reject them. Rejection itself was brought about by a second step which was thought to be non-specific and effective against damaged, but not undamaged worms. In several respects Ogilvie and Jones detailed suggestion proved to be incomplete, but study of the "two-step" principle in a number of laboratories has led to the realization that synergy encompasses both humoral and cellular components of the immune system. Keller and Keist (1972) showed that antibodies alone could not cause rejection of damaged *N.brasiliensis*, but that rejection did occur in the presence of sensitized lymphocytes. Therefore the theory proposed by Ogilvie and Jones (1971) was adapted to include the requirement of specifically primed "cells" in addition to antibody. Confirmation that the cells involved in the

*Nippostrongylus brasiliensis* system were T-lymphocytes was provided by Ogilvie *et al.* (1977). A similar conclusion was reached by Crum *et al.* (1977) for *Trichinella spiralis*.

Thus it seems clear that in certain systems, at least, cells and antibodies interact to cause worm expulsion. In the light of present day knowledge of the immune system, this principle presents us with a complex of possible pathways. Figure 1:1, page 3, shows a simple diagrammatic representation of the basic reactions now thought to occur during the host's anti-worm response. Initially parasite antigen interacts with lymphocytes to cause the production of specific antibody and activation of specific T cells (Fig.1:2, page 4). The next step in the host's response seems to depend on the particular parasite involved, since the location of the worm will to some extent control which effector mechanisms can actually reach the organism, and the type of antigens produced by the parasite probably vary with species. The following is a summary of the possible systems that may be recruited during an immunologically mediated rejection of the parasite population.

### 1.1. Antibodies

Originally it was suggested that antibodies had a purely mechanical function, since antigen-antibody precipitates were demonstrated *in vitro* at oral and anal pores (Sarles, 1938; Taliaferro and Sarles, 1939). This led to the suggestion that these complexes blocked the feeding of the parasite and caused its death by starvation. This is rather a crude idea and no one has demonstrated that such plugs have a detrimental effect on worm survival.

An alternative function was proposed for antibodies, as effectors in the "leak lesion" hypothesis suggested by Urquhart (1965). It was proposed that

Fig.1:1

The possible steps during an immune response against a nematode parasite

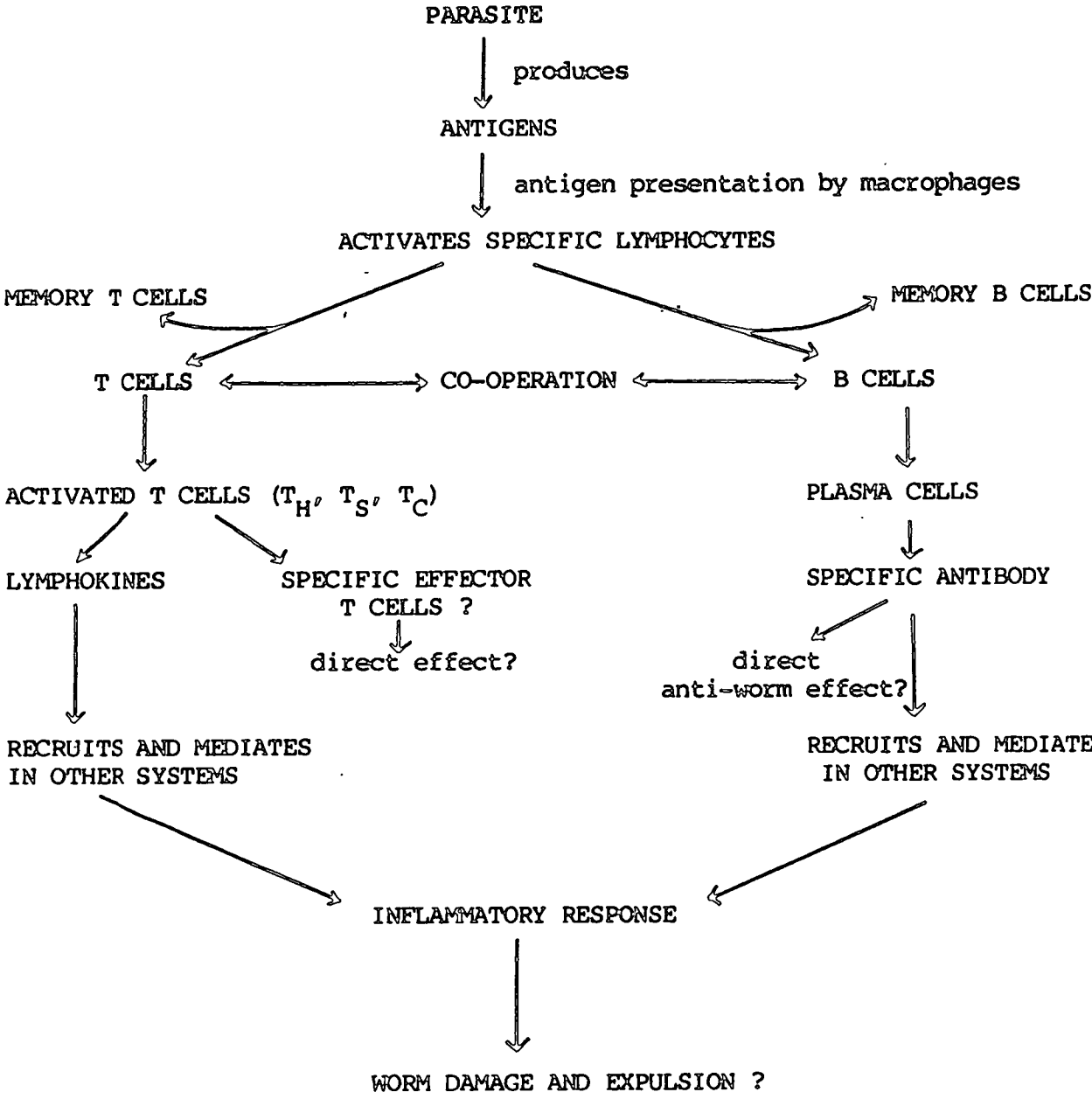
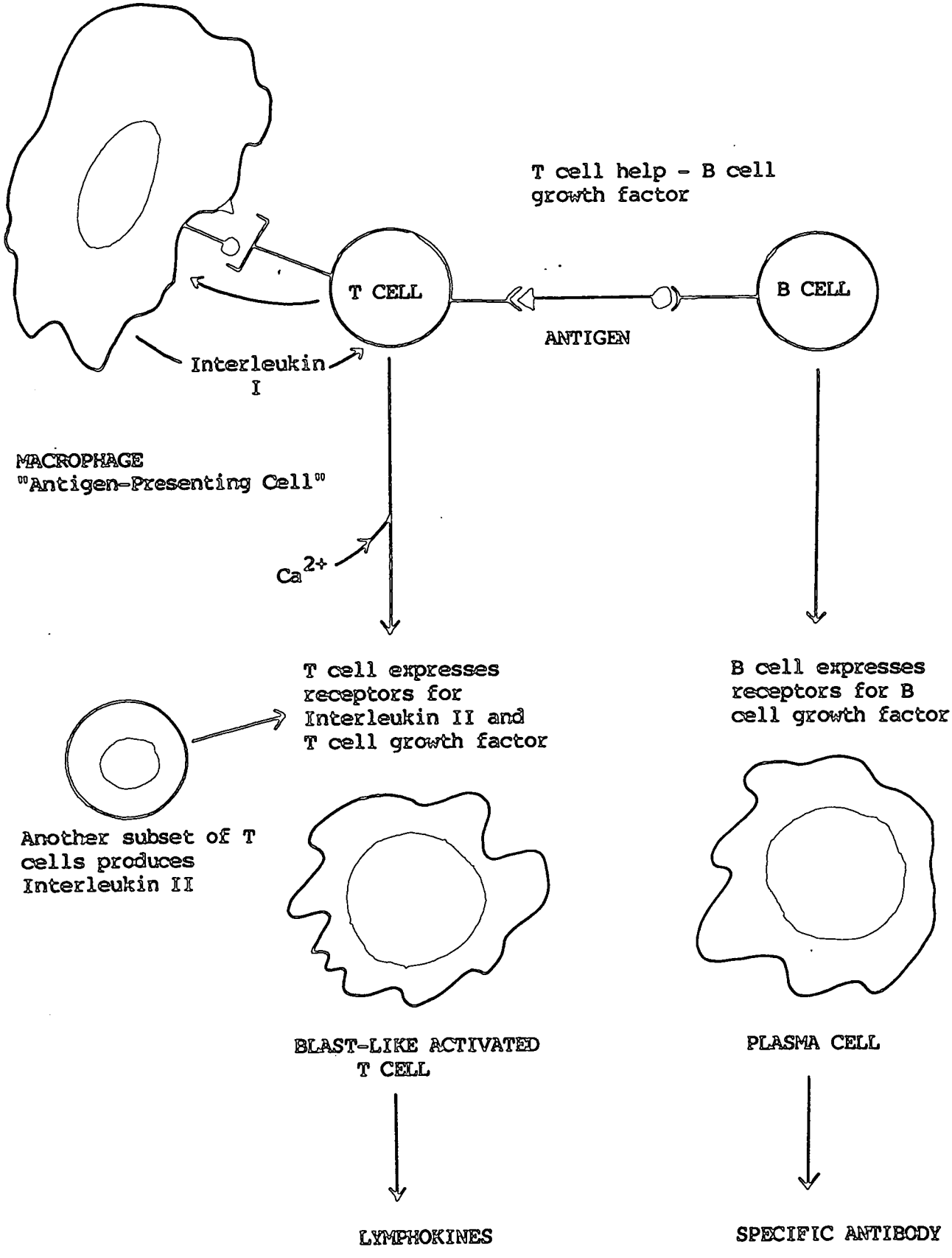


Fig.1:2

## Activation of lymphocytes



specific anti-worm reagins, (now known to be immunoglobulin E's), sensitised mast cells and then the interaction of the worm antigen with the IgE led to the degranulation of mast cells, the release of vasoactive amines, and a local anaphylaxis. This increased the traffic of more parasite- specific antibody (mainly immunoglobulin G) to the site of the infection, which caused worm damage and expulsion. In support of this theory Barth (1966) showed that an anaphylactic response induced to an unrelated antigen did potentiate the effect of antiserum and enhanced the rejection of *N.brasiliensis* compared with unshocked controls. However, Ogilvie (1967) repeated the experiments of Barth using an adult *N.brasiliensis* antigen to cause a specific anaphylactic response, but it was incapable of producing worm expulsion. Thus although the leak lesion hypothesis could explain results within a particular experimental framework, that framework was revealed to be an inadequate representation of real host parasite relations.

Classically, IgE has been the main immunoglobulin class associated with helminth infections but only a relatively small proportion has been found to be parasite-specific (reviewed by Jarrett, 1978). IgG seems to be the main class responsible for protection. Thus the major role of antibody in an immune response seems to be one of mediation, since IgG is capable of increasing the effectiveness of other non-specific effector mechanisms, and it can bring these components into close contact with the parasite. Despite a number of attempts to incorporate IgE into a theoretical framework, the actual role of this class of antibody produced during infection remains unknown. It may be selectively induced by the worm to hinder an effective immune response since the non-specific IgE may interfere competitively with the binding of parasite-specific IgE to mast cells, eosinophils or macrophages and thus restrict the amount of damage which could be inflicted by these cells on the

parasites (Jarrett and Miller, 1982).

### 1.2. T cells

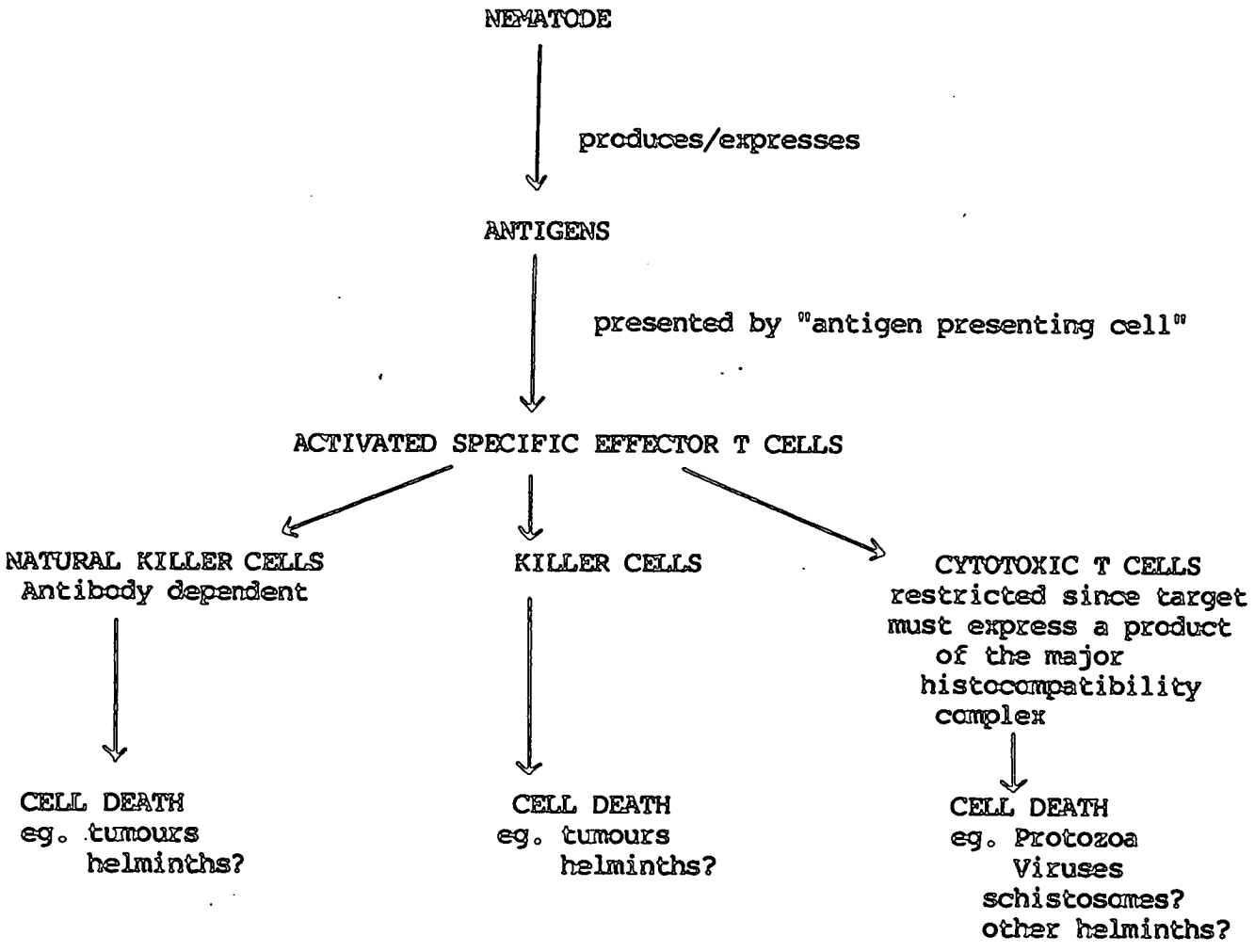
Theoretically T lymphocytes should be capable of damaging nematodes, but the evidence suggests that this is unlikely (Fig.1:3, page 7), for the type of reactions which could occur.

The action of cytotoxic T cells is restricted since targets must express a "self-molecule", a product of the major histocompatability complex, as well as foreign antigenic determinants. There is no logical reason why nematodes should express such a molecule, but it has been shown that, among the platyhelminths, the schistosomes do acquire a variety of host derived molecules on their surface (Smithers *et al.*, 1969; Clegg *et al.*, 1971), including products of the major histocompatibility complex (Sher *et al.*, 1978). It has been demonstrated *in vitro* that cytotoxic T cells attach to and damage schistosomes under certain conditions (Ellner *et al.*, 1982), but there is no similar evidence for nematodes. Natural Killer and Killer cells are not similarly restricted, but little is known about their function. So far *in vitro* experiments using these cells have been negative (Vadas *et al.*, 1979a; Mackenzie *et al.*, 1980, 1981; Greene *et al.*, 1981), implying that they are not involved in worm expulsion. Therefore the major role of T cells seems to be analagous to that of antibodies, that is, primarily one of mediation.

### 1.3. The macrophage

The main role of the macrophage in nematode immunity also seems to be one of mediation, as the "antigen-presenting cell" (Fig.1:2, page 4), although it appears to have an active role in killing other parasites, such as intra-cellular

Fig.1:3 T cells as effectors



viruses and protozoa (Fig.1:4, page 9). *In vitro* studies have shown that macrophages are capable of damaging microfilariae of *Litomosoides carinii* (Mehta *et al*, 1980), and *Dipetalonema viteae* (Haque *et al*, 1980), but there is no evidence that they damage adult worms. They are probably involved in clearing damaged worms from the body, and perhaps they have an effector role against the migratory larval stages of the parasite.

## 1.4. The mast cell

The importance of the mast cell as an effector was suggested as part of the "leak lesion" hypothesis of Urquhart (1965). Since then its function in nematode rejection has been studied in detail (Fig.1:5, page 10) for its possible effects. In rats infected with *Trichinella spiralis*, mucosal mastocytosis and degranulation of the cells has been demonstrated to be temporally associated with the immune expulsion of the adults worms (Alizadeh and Wakelin, 1982a). A similar increase in the number of mast cells over the course of infection has also been shown in rats infected with *Strongyloides ratti* (Moqbel, 1980; Mimori *et al*, 1982), implying that the cells are important in parasite rejection. It was originally thought that mast cells acted as effectors in the expulsion of *N.brasiliensis* from rats since a similar increase in mast cell number has been observed (Taliaferro and Sarles, 1939). However cell degranulation often did not occur until parasite expulsion was completed, and lactating rats, which show mast cell infiltration at the same time and to the same extent as non-lactating controls, are unable to expel their worms. Recently, Woodbury *et al.* (1984) have shown that the systemic release of mast cell protease II, specifically from mucosal mast cells, coincides with the immune rejection of *N.brasiliensis* and *T.spiralis*, implying that it has a functional role in both systems. However, secretion of this enzyme occurs in the absence of

Fig.1:4 The role of the macrophage in nematode immunity

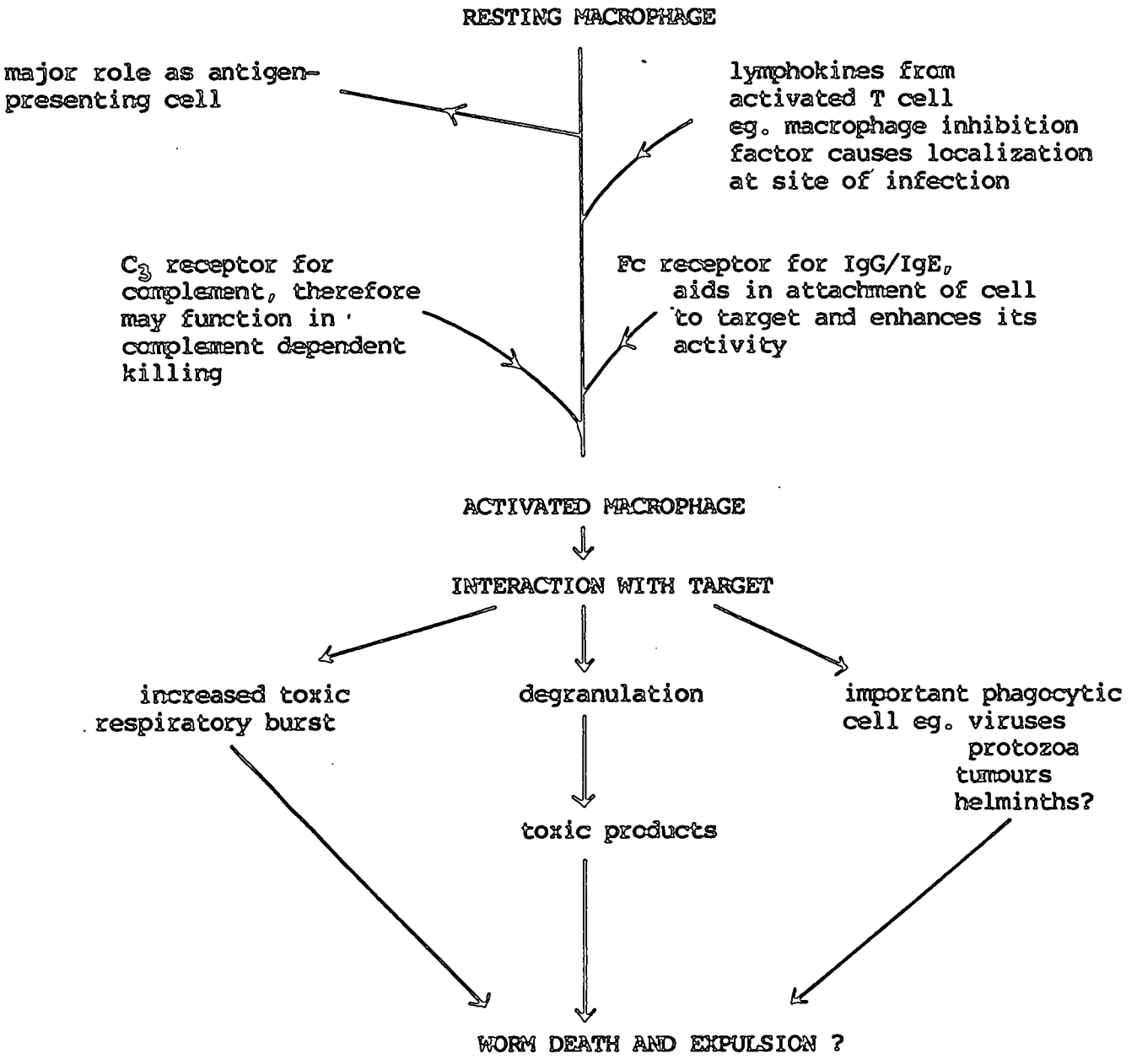
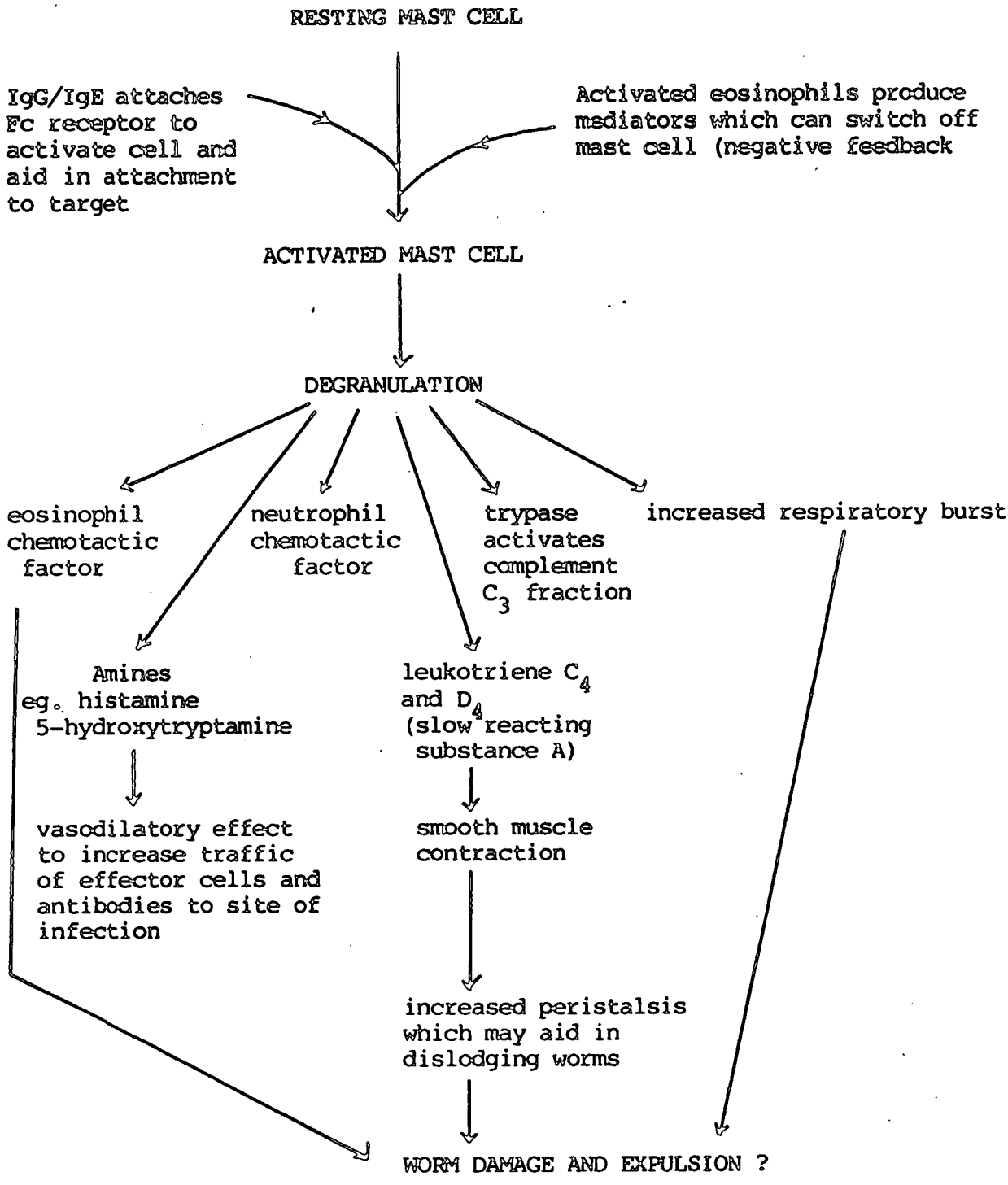


Fig.1:5 The role of the mast cell in nematode immunity



detectable parasite-specific IgE.

### 1.5. The eosinophil/ neutrophil/ basophil

Other cells which may have an effector role in parasite rejection are the eosinophil, the neutrophil and the basophil (Figs.1:6, 1:7 and 1:8, pages 12, 13 and 14), for their possible actions in worm expulsion.

A rise in the number of tissue eosinophils within the lamina propria of the small intestine during the course of infection has been demonstrated in rats infected with *S.ratti* (Moqbel, 1980), *N.brasiliensis* (Taliaferro and Sarles, 1939; Wells, 1962; Kelly and Ogilvie, 1972), and *T.spiralis* (Race *et al*, 1974). It has also been shown in mice infected with *T.spiralis* (Ruitenberg *et al*, 1977), thus leading to the suggestion that these cells were involved in the host's anti-worm response. *In vitro* studies have shown that eosinophils are capable of killing infective larvae of *Dictyocaulus viviparus* (Knapp and Oakley, 1981), and microfilariae of *Dipetalonema viteae* (Mehta *et al*, 1981b). Moqbel (1980) found that eosinophils were in close contact, and perhaps even adhered to, infective *S.ratti* larvae present in the skin of rats that were hyperimmune, implying that they may be functional in the host's immune response against the parasite.

Neutrophils have also been suggested as important effectors. They can kill a number of targets *in vitro*, such as microfilariae of *L.carinii* (Hopper *et al*, 1981), and schistosomula of the trematode, *Schistosoma mansoni* (McLaren *et al*, 1981), but there is little evidence to implicate them *in vivo*.

Basophils are thought to be important in the rejection of the sheep nematode *Trichostrongylus colubriformis* from guinea pigs (reviewed by

Fig.1:6 The role of the eosinophil in nematode immunity

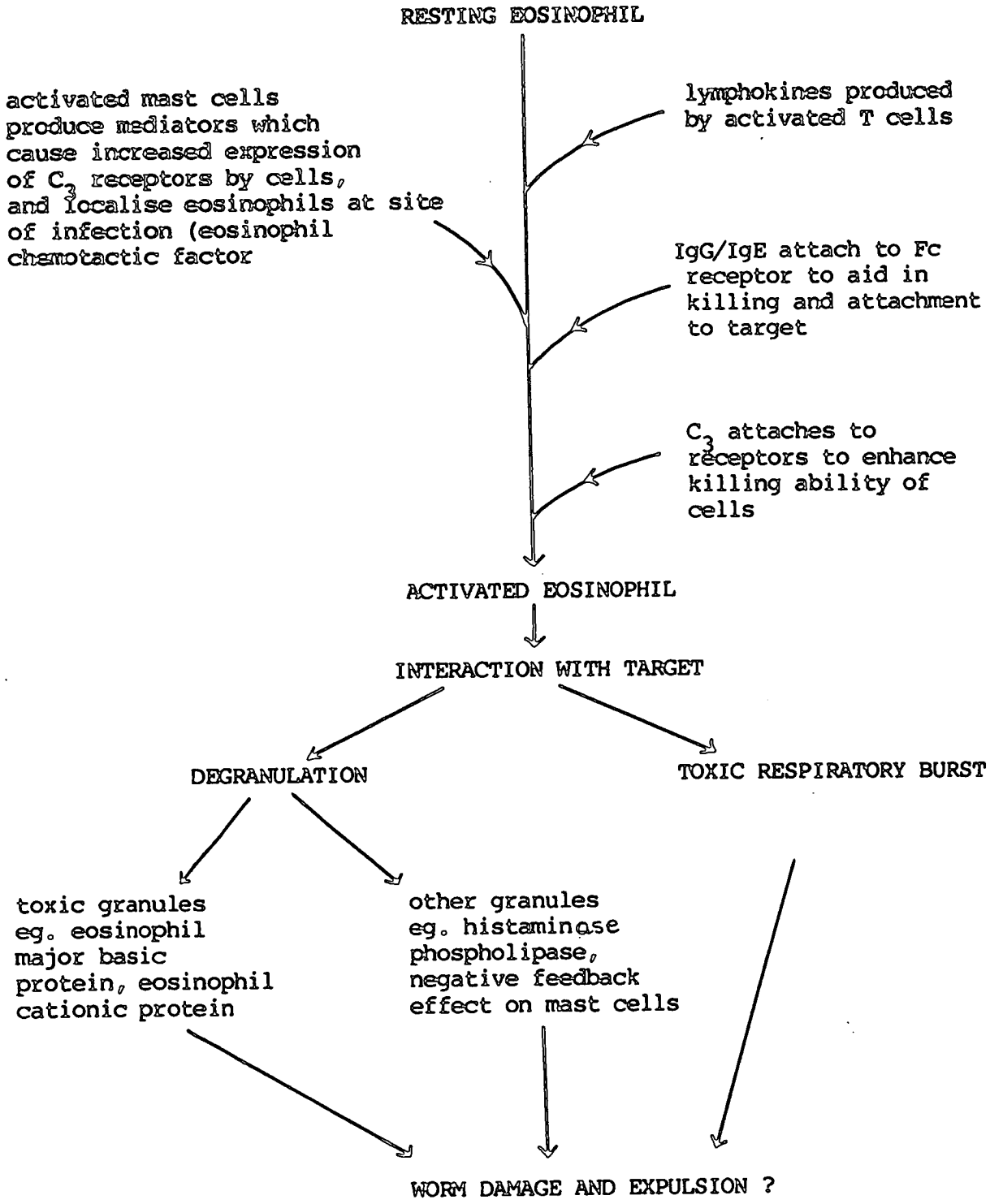


Fig.1:7 The role of the neutrophil in nematode immunity

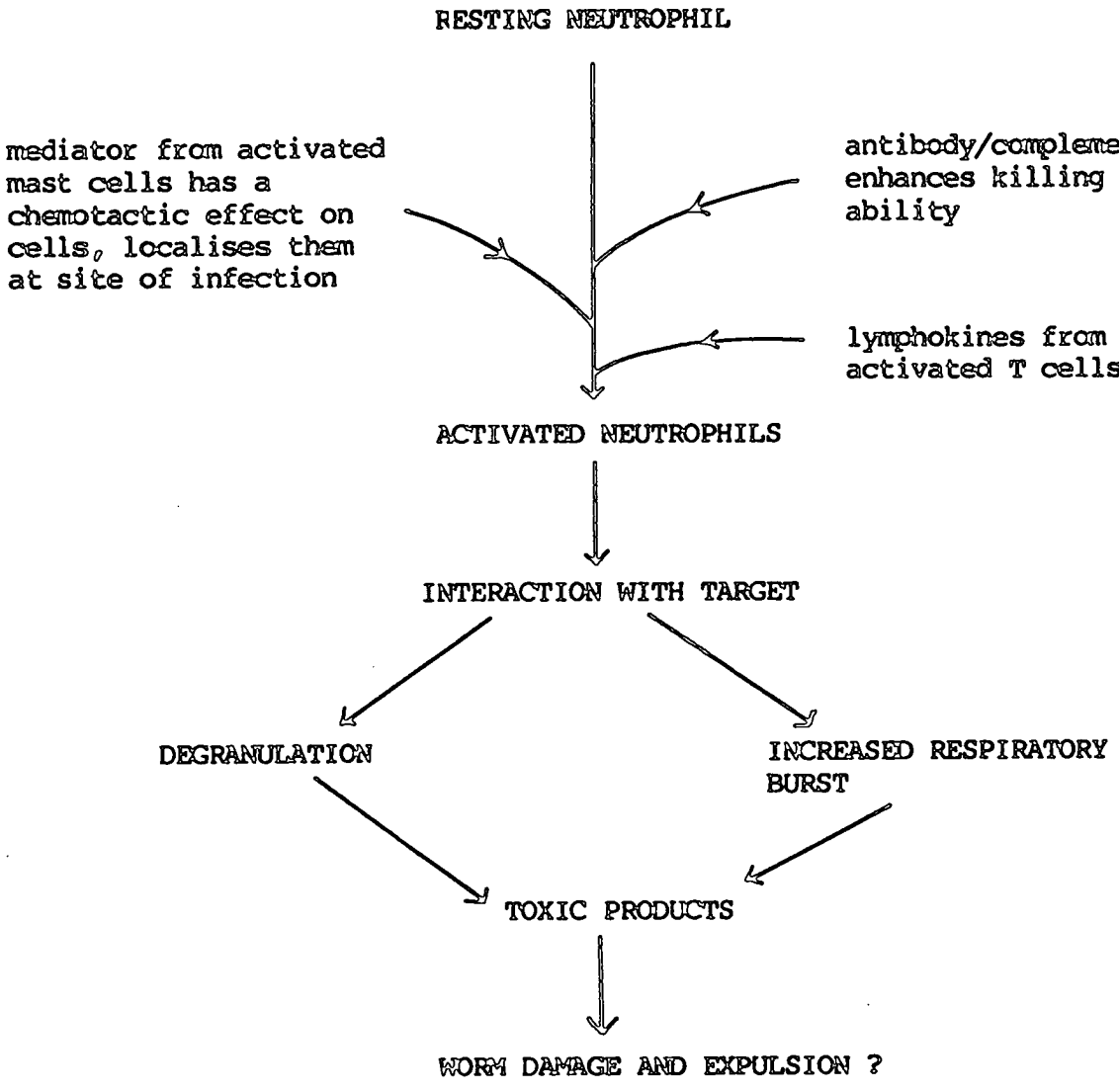
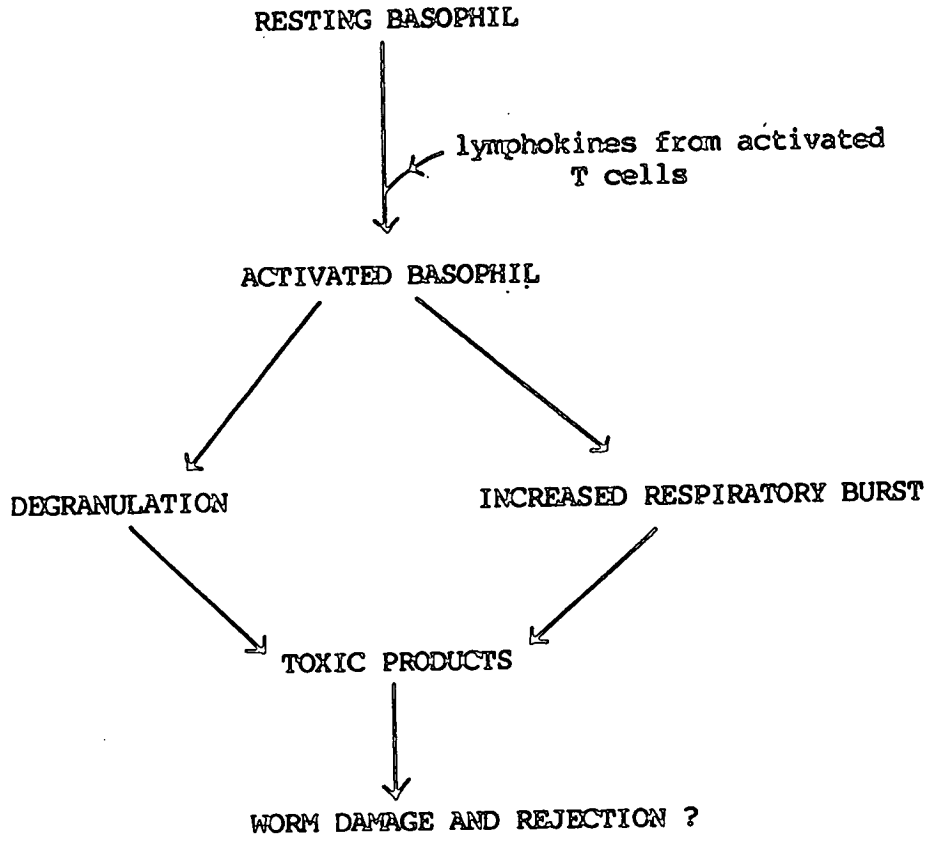


Fig.1:8 The role of the basophil in nematode immunity



Askenase, 1977), since basophil infiltration of the intestine correlates well with the course of infection. However there is little evidence to suggest that they have an active role in other, more natural, host/parasite systems.

### 1.6. Other possible effector mechanisms in worm rejection

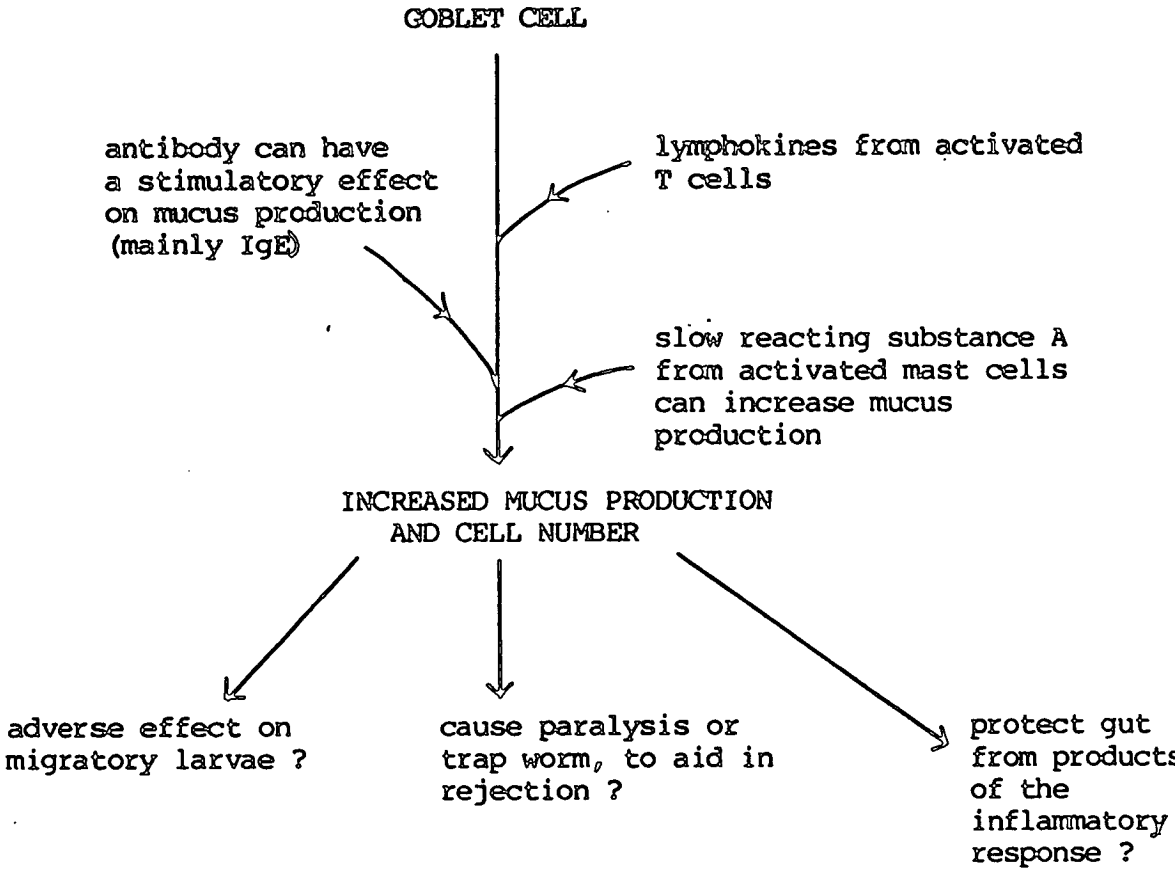
A variety of other non-specific responses have been implicated as having a role in the host's anti-worm response. An increase in the number of mucus-secreting cells and in the amount of mucus secreted into the intestine, has been demonstrated in many parasite infections (Miller *et al.*, 1982, Lee and Ogilvie, 1981). It has been suggested that mucus could have a direct anti-worm effect (Miller *et al.*, 1981) by excluding the parasite from its preferred site, affecting the worm's respiration, or possibly paralysing it, since mucus contains substances which have the same physical properties as leukotriene $C_4$ and $D_4$ (slow-reacting substance A) which causes smooth muscle contraction (Fig.1:9, page 16). The increased mucus production could however be purely a host protective response, to prevent the intestinal wall from being damaged by the mediators released during inflammatory responses.

An increase in the level of gut motility has also been demonstrated in rats infected with *N.brasiliensis* (Farmer, 1981), and it has been shown that infection of rats with *T.spiralis* causes an increase in the amount of fluid secreted into the intestine (Castro *et al.*, 1979). Both of these manifestations could assist in dislodging adults during worm rejection.

### 1.7. Parasite antigens

The parasite antigens responsible for eliciting an immune response in the broadest terms have been divided into two main types, either

Fig.1:9 The possible role of mucus in parasite expulsion



excretory/secretory or somatic. It is relatively simple to demonstrate that preparations of either one are antigenic, but it is difficult to pinpoint the substances which act as functional antigens. On the whole, most of the evidence implies that the excretory/secretory antigens are the most active, since immunization with somatic preparations often fails to protect the host against re-infection even though specific antibody against them can be demonstrated (reviewed by Clegg and Smith, 1978; and Llyod, 1981). It has been shown that the cuticular antigens of many parasites, such as *N.brasiliensis* (Maizels *et al*, 1982), and *T.spiralis* (Mackenzie *et al*, 1978; Philipp *et al*, 1980b; Parkhouse *et al*, 1981), show stage specificity. Therefore perhaps successful immunization requires priming with a preparation containing antigens expressed by all parasitic stages of the worm. However the only commercial vaccines produced against nematode parasites, that is, against *Dictyocaulus viviparus* in cattle, *Dictyocaulus filaria* in sheep and *Ancylostoma caninum* in dogs, are theoretically based on the ability of the irradiated larvae to produce metabolic antigens *in vivo* (reviewed by Peacock and Poynter, 1981).

In experimental systems excretory/secretory antigens can be potent inducers of a protective response, but how valid is such a distinction? Stichosome antigens derived from the trichuroid nematodes, *T.spiralis* and *Trichuris muris*, have been shown to be very immunogenic and protective (Despommier, 1981; Jenkins and Wakelin, 1977), and analysis of the proteins from the granules demonstrated the presence of several groups of antigens (Despommier, 1981) which were identical to those secreted by the larvae in culture (Despommier and Muller, 1976). This observation calls into question the categorisation of parasite products into two groups, since excretory/secretory and somatic preparations may possess the same epitopes.

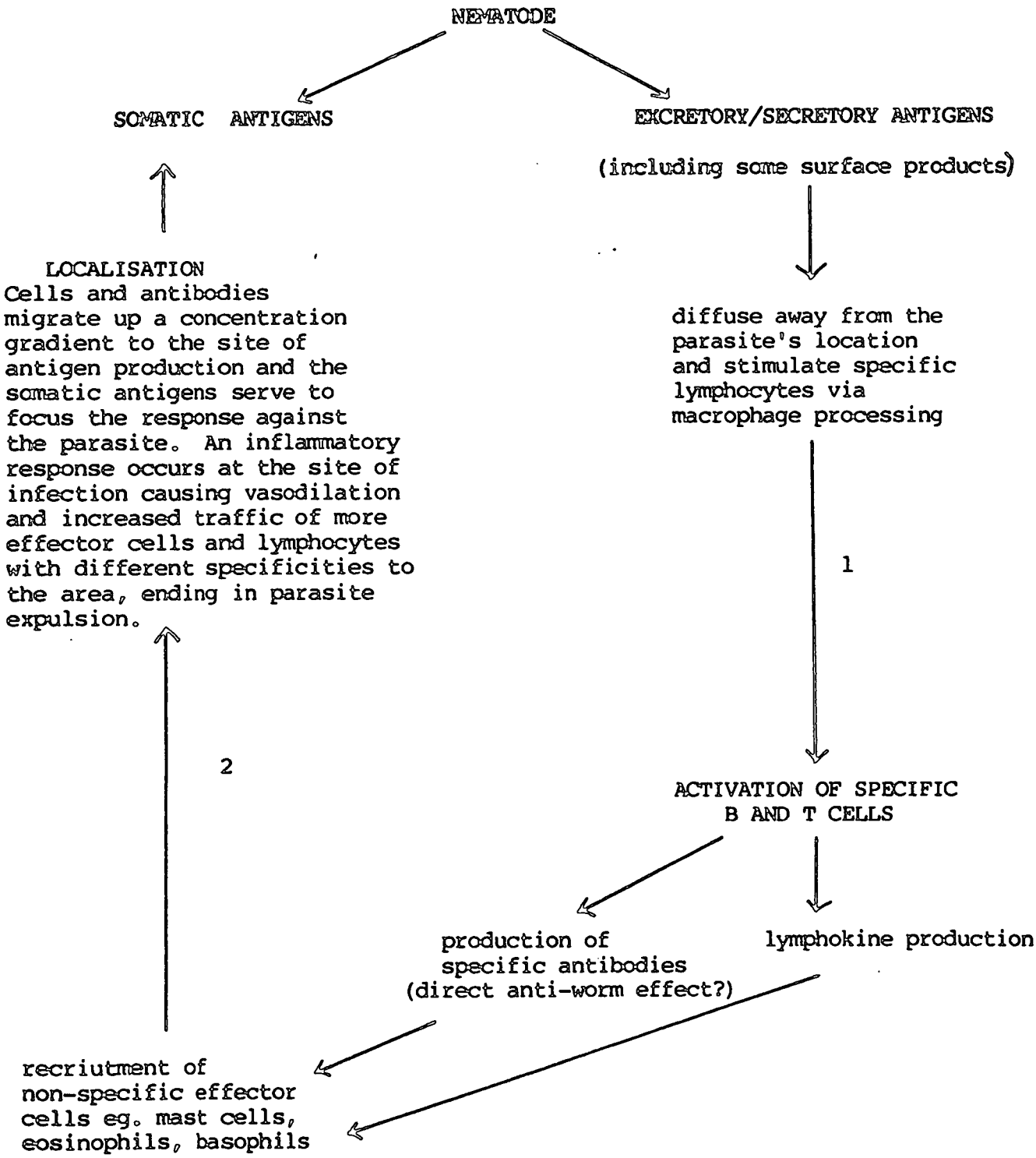
Discarding crude ideas such as those referred to earlier (Section 1, page 2) it is difficult to envisage how excretory/secretory antigens could be protective in any simple way, since once produced they probably diffuse away from the vicinity of the parasite. Perhaps their effect is one of localising the immune response and directing the effector mechanisms against the nematode, with the "somatic" antigens being targets involved directly in worm damage. Thus the two types of antigen, if they are so described, may act in synergy (Fig.1:10, page 19½, which shows the reactions which could occur). It has been demonstrated that *T.spiralis* sheds surface antigens when cultured *in vitro* (Philipp *et al*, 1980), therefore it is possible that this occurs *in vivo*. Thus cells recruited against excretory/secretory antigens, could also contain a proportion of lymphocytes with specificities for somatic antigens, which would also migrate up the concentration gradient to the site of antigen production, and be able to direct specific effector mechanisms against the worm, and mediate in worm damage by non-specific components of the immune system.

## 1.8. The role of specific and non-specific components of the host's immune response against nematode parasites

Once it was apparent that cellular and humoral components acted in synergy to cause worm rejection research began to be directed towards discovering the relative contribution of specific and non-specific mechanisms in bringing about this effect. One approach to this question, adopted by a number of workers, was to attempt to define the specificity of the response by studying cross-resistance against different sorts of parasite.

When parasites are present together in a host no consistent picture emerges. Concurrent infection with a nematode and a virus, or bacterium,

Fig.1:10 The possible reactions produced by parasitic somatic and excretory/secretory antigens



often leads to the enhanced survival of the micro-organism, possibly because there is competition for effector mechanisms. In other systems the increased survival of one of a pair may be because its partner is capable of causing immunosuppression. Mice, concurrently infected with *Heligmosomoides polygyrus*(*Nematospiroides dubius*) and another helminth produce a diminished response to the second species (Behnke *et al*, 1978 for *T.spiralis* and *N.dubius*; Hopkins, 1980 for *Hymenolepis diminuta* and *N.dubius*; and Jenkins, 1975 for *N.brasiliensis* and *N.dubius*). It is thought that *N.dubius* produces immunomodulatory factors, believed to be excretory/secretory antigens, which somehow induce the production of suppressor T cells, causing a general "switching off" of the host's immune response.

In contrast, concurrent infection can also enable the host to express increased resistance. For example, infection with *T.spiralis* increased the resistance of mice to the intra-cellular bacterium, *Listeria monocytogenes* (Cypess, 1974), and to the protozoan parasite *Plasmodium berghei* (Ngwenya, 1982). This was thought to be due to an increase in systemic cellular immunity brought about by the nematode infection. Similarly, if mice are infected with *T.spiralis* during the acute phase of the inflammatory response against an existing *A.caninum* infection, there is a reduction in the number of adult *T.spiralis* recovered compared to controls (Goulson, 1958). In this case it was thought that the inflammatory response elicited against one parasite is acting non-specifically against the other.

Probably a better system in which to investigate the basic question is one designed to test the specificity of the immunological memory. This can hopefully be done by sequential infections of the different parasites. Within this sort of framework protozoan parasites seem to generate strict

species-specific immunity, for example, cross-resistance studies using non-concurrent infections of different species of the coccidean genus, *Eimeria*, showed there was little or no cross-immunity induced (Becker *et al*, 1933; Tyzer, 1929; Levine, 1938; Moore and Brown, 1951). At a finer level, there is some evidence for the existence of intra-specific cross-immunity from studies using two strains of *E.acervulina* (Joyner, 1969). In contrast, studies with nematode parasites suggest that inter-specific cross-immunity is the general rule. Some degree of reciprocal cross-immunity has been demonstrated for the following pairs of parasites; *S.ratti* and *N.brasiliensis* in rats (Nawa *et al*, 1982), *T.spiralis* and *S.ratti* in rats (Moqbel and Wakelin, 1979), and *Trichuris muris* and *T.spiralis* in mice (Lee *et al*, 1982). These vary in their morphology, inhabit different locations in the gastro-intestinal tract, and have a different biology, compared with their respective partner. However in all cases homologous challenge produced a higher level of protection, demonstrating that the host could differentiate between the two parasites.

No one has set out to discover the limit of the host's ability to distinguish between parasites, which could be vital since it might provide a framework in which the contribution of specific and non-specific components of the immune system in worm damage and rejection could be explored. Some work has been carried out comparing the host's response to closely related worms, by using parasites from the same genus; for example, reciprocal cross-immunity has been shown between *Trichostrongylus colubriformis* and *Trichostrongylus vitrinus* in sheep (Dineen *et al*, 1977) and *Cooperia oncophera* and *Cooperia pectinata* in calves (Herlich, 1965). More recently, Dawkins and Grove (1982), showed that mice primed with *Strongyloides stercoralis* expressed some resistance against *S.ratti*, and Coop *et al.* (1986, in press) showed that prior infection of lambs with *Ostertagia ostertagi* conferred some

protection against heterologous challenge with *Ostertagia circumcincta*, but in neither study was the reciprocal challenge experiment carried out. Moreover, both *Strongyloides spp.* and *O.circumcincta* were in an unnatural host.

There seem to be no studies on the existence of reciprocal intra-specific cross-immunity between nematode parasites, which would be a real test of the host's ability to differentiate between worms.

In the following experiments this aspect of nematode immunology was investigated, using two strains of *S.ratti*, and *S.venezuelensis*. Since these parasites occur naturally in the rat it was anticipated that there would be no problem in carrying out reciprocal challenge experiments, and in interpreting cross-reactions in a consistent quantitative framework. *S.ratti* and *S.venezuelensis* parasitic female worms can be differentiated using a morphological characteristic; adults of the two *S.ratti* strains are structurally indistinguishable. The strains differ in the type of development they express during the free-living stages of their life cycle. In assaying for small changes in the host's immune response as precise an experimental system as possible was used having the minimum error variance. Throughout the study, therefore, rats were infected with small "exact" doses of infective *Strongyloides* larvae, using a method pioneeered in this laboratory. As many relationships as possible were investigated, in terms of priming and challenge with the three parasites, using a variety of immunological parameters.

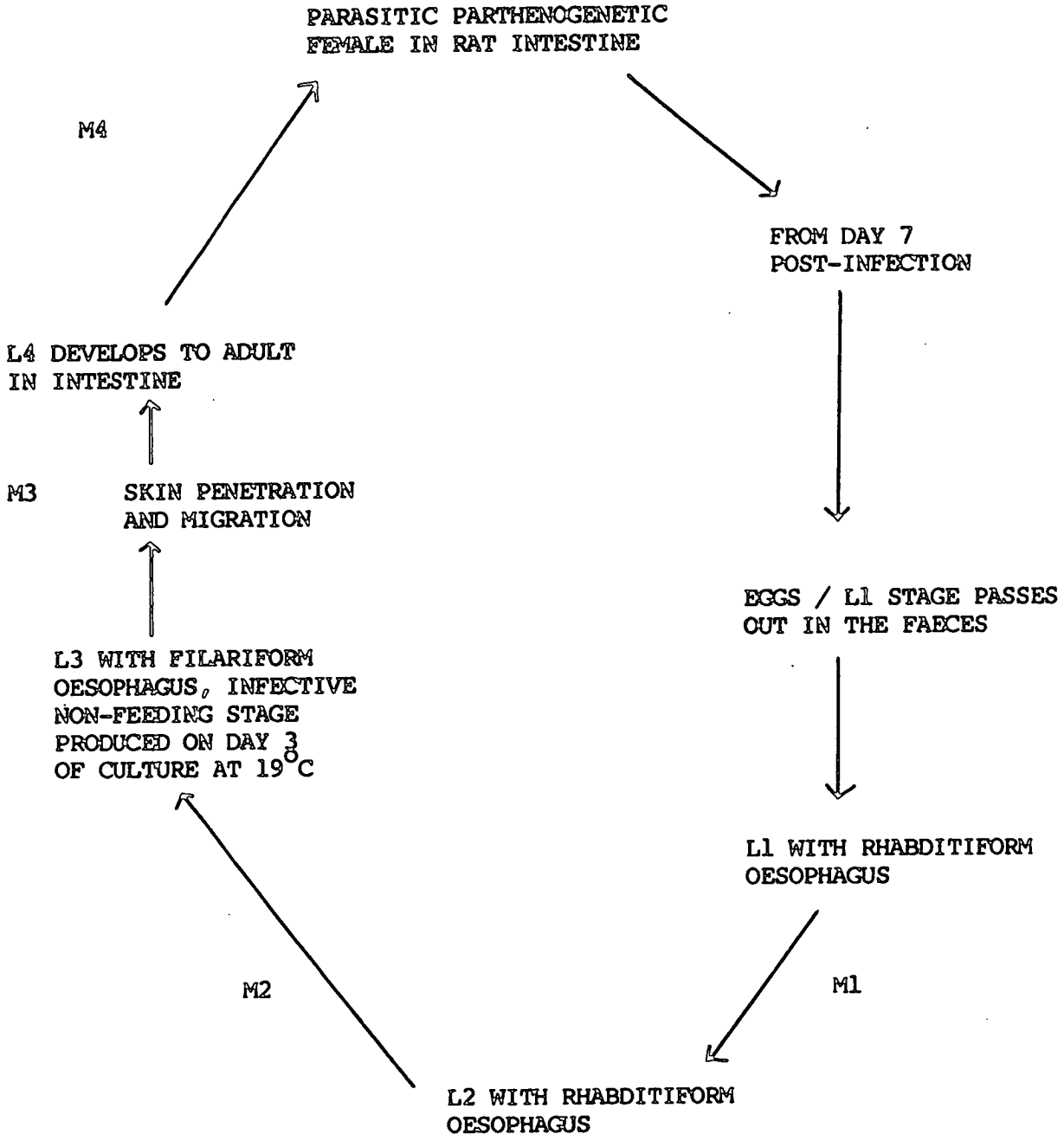
CHAPTER 2

GENERAL MATERIALS AND METHODS

## 2.1. Parasites used in study

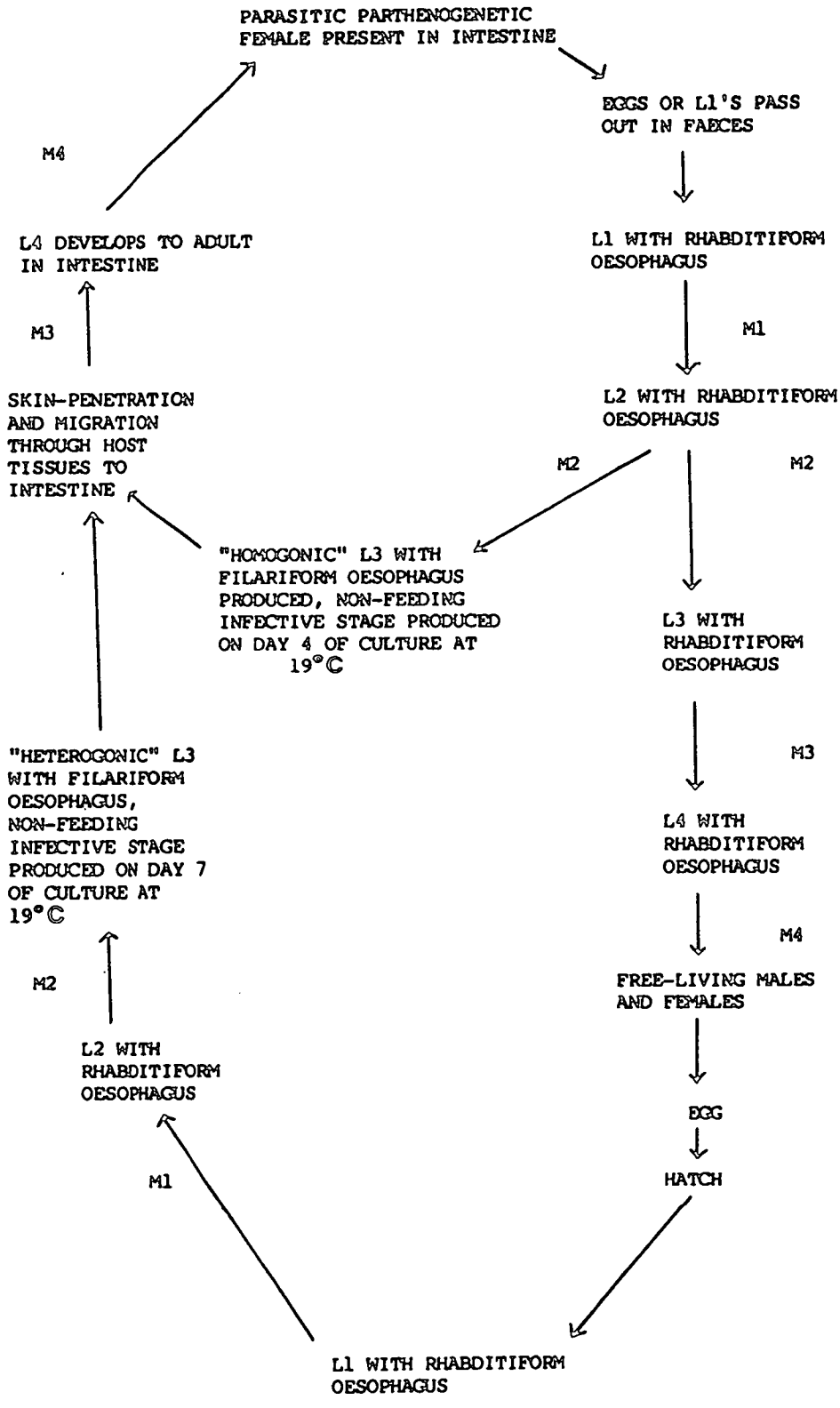
*Strongyloides venezuelensis* (Brumpt, 1934) and two strains of *Strongyloides ratti* (Sandground, 1925), designated the "homogonic" and the "heterogonic" strain were maintained by weekly passage. The "homogonic" strain was originally obtained from the Wellcome Laboratory, (Langley Court, Beckenham, Kent) in the 1960's; however over the years there have been various replacement stocks, which were also believed to have originated from Wellcome. The "heterogonic" strain was obtained in 1973 from Professor Guta Wertheim, (Jerusalem, Israel). It was originally isolated from a wild rat in Philadelphia, USA, by Dr. G. Graham in 1960, and became known as the G-60 strain. The *S.venezuelensis* stock was obtained specifically for this study from Dr. F.Katz (Seton Hall University, South Orange, New Jersey, USA), in February 1983 and August 1984. He originally obtained it from Professor G.Wertheim in Israel. The two *S.ratti* strains are differentiated by their type of life cycle (Figs.2:1 and 2:2, pages 24 and 25), since the "heterogonic" biology includes free-living males and females, morphologically distinct from the parasitic parthenogenetic females (Fig.2:3, pages 26-29) and absent from the cycle of the homogonic strain. *S.venezuelensis* is reported to have a "heterogonic" type of life history (Fig.2:2, page 25) - personal communication from Dr. F.Katz to Dr. P.A.G.Wilson - but the "homogonic" phase seems to predominate, since no free-living males and females have been seen in cultures in this laboratory. Parasitic female worms of the two *Strongyloides spp.* could be distinguished using a morphological characteristic since the uterus and intestine of *S.ratti*

Fig. 2:1
The life cycle of homogonic S.ratti




Where:-
M = a moult
L = a larval stage

Fig. 2:2
The life cycle of heterogonic S.ratti




where:-
M = a moult
L = a larval stage

Fig.2:3a Parasitic parthenogenetic female *S.ratti* worm recovered from the host's intestine. Note that the worm has a straight intestine and uterus (size approximately 3mm).

Fig.2:3b Posterior end of female parasitic *S.venezuelenis* worm. Note spiralling of intestine and uterus (size approximately 0.4mm).

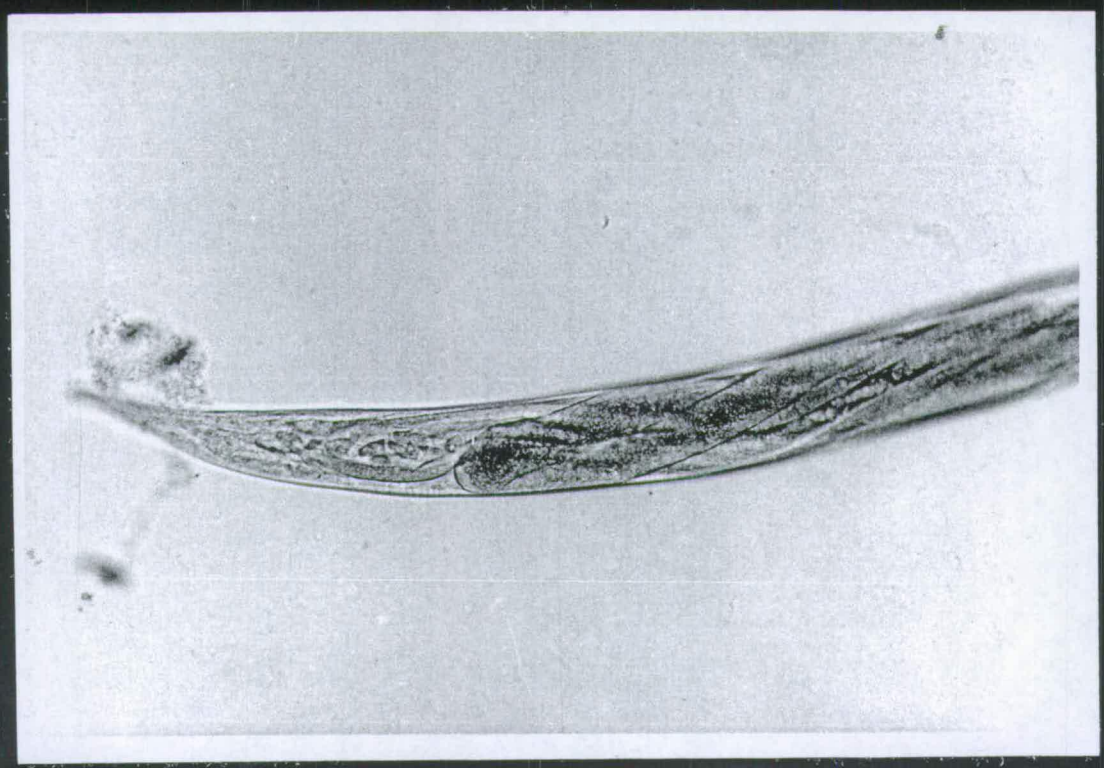


Fig.2:3c Free-living female heterogonic *S.ratti* worm from cultured faeces (centre, size approximately 2mm). Note how the appearance differs from the parasitic female shown in (a).

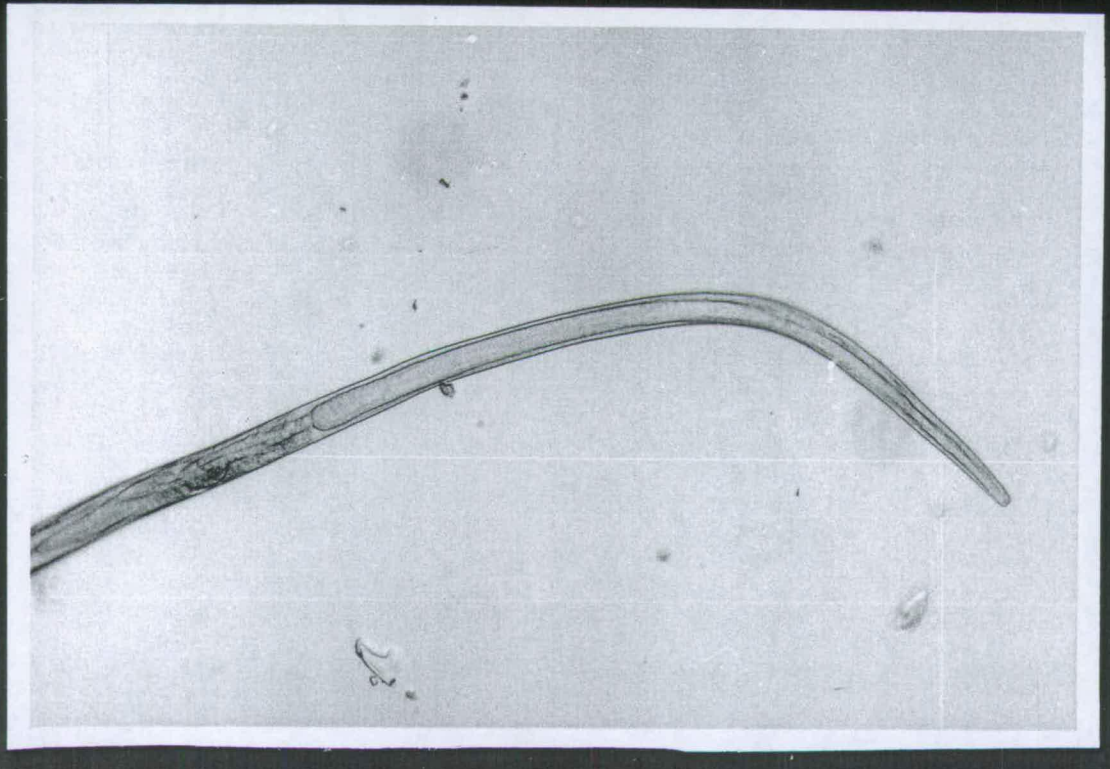
Fig.2:3d Free-living male heterogonic *S.ratti* worm from cultured faeces. Spicules are present at the curved posterior end of the worm (size approximately 1.5mm).

Fig.2:3e *S.venezuelensis* eggs from a fresh faecal smear. The eggs do not contain a fully formed first-stage larva (size 40-70μm).

Fig.2:3f Eggs of *S.ratti* in section of the small intestine. The eggs already contain the first-stage larva which will probably hatch out of the egg before it passes out in the faeces (size 40-70μm).

Fig.2:3g Filariform oesophagus of parasitic *S.ratti* female worm (size approximately 0.5mm).

Fig.2:3h Rhabditiform oesophagus of free-living heterogonic *S.ratti* female worm (size approximately 0.3mm).

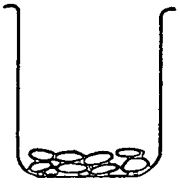


parasitic females are straight, whereas those of *S.venezuelensis* are coiled around each other (Fig.2:3a and b, page 26). There was also a difference in the type of development exhibited by the egg of the two species. Eggs of *S.ratti* contain a fully developed first- stage larva and often hatch before they pass out of the host; whereas those of *S.venezuelensis* are still in the early stages of cleavage when they pass out of the host.

The culture method for both species was essentially the same (after Wilson *et al*,1978b). Rats with stock infections were held in cages with grid bottoms suspended over trays lined with moist paper towelling which was replaced daily. Starting on day 7 of an infection, overnight faeces were collected, checked by microscopy for patency, and then rinsed with distilled water to limit urine contamination. Approximately 8-12 faecal pellets were placed on a watch-glass, which was transferred to a glass Petri-dish. Distilled water was added to the faeces and under the watch-glass, before replacing the Petri-dish lid (Fig.2:4, page 31). The cultures were maintained for 3 days for "homogonic" *S.ratti* and *S.venezuelensis*, and 7 days for "heterogonic" *S.ratti* in an anhydric incubator with an internal circulation of air (Vindon Scientific Ltd.) at 19°C ± 1°C for *S.ratti* and 25°C ± 2°C for *S.venezuelensis*. Usually six Petri-dishes were set up on any day and on average 4000 third-stage larvae were harvested per dish. After 48 hours distilled water was added to the faeces of *S.venezuelensis* and "homogonic" *S.ratti* cultures, and infective filariform larvae were collected 24 hours later. In the case of "heterogonic" *S.ratti* cultures distilled water was added to the faeces on days 3 and 6, the larvae which emerged on day 4 (i.e. the "homogonic" larvae) being removed and discarded. The truly "heterogonic" *S.ratti* larvae were harvested on day 7. Material collected from all dishes, was pooled, and washed by centrifugation (approximately 500g, MSE.) in a 10ml graduated glass tube (MSE.), then

Fig. 2:4

The faecal culture method



FAECES WERE COLLECTED OVERNIGHT AND HARVESTED THE NEXT DAY

1

A FAECAL SMEAR WAS CARRIED TO CONFIRM INFECTION MICROSCOPICALLY

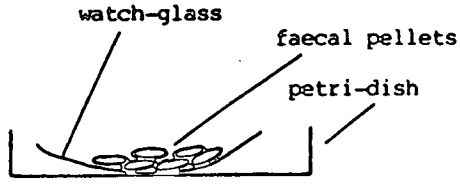
S.ratti - L1 / EMBRYONATED EGGS SEEN IN FRESH SMEAR

S.venezuelensis - ONLY UNEMBRYONATED EGGS SEEN IN FRESH SMEAR

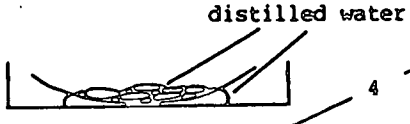
2

THE FAECES WERE WASHED IN DISTILLED WATER TO LIMIT URINE CONTAMINATION

3




A FEW PELLETS WERE PLACED ON A WATCH-GLASS WHICH WAS PUT INTO A PETRI-DISH. A SMALL AMOUNT OF DISTILLED WATER WAS ADDED TO THE FAECES AND UNDER THE WATCH-GLASS TO PROVIDE HUMID CONDITIONS




4

THE PETRI-DISH WAS MARKED WITH THE STRAIN/SPECIES AND INCUBATED AT THE REQUIRED TEMPERATURE.

5

AFTER THE REQUIRED TIME THE WATER UNDER THE WATCH-GLASS CONTAINING INFECTIVE LARVAE WAS COLLECTED. THE NUMBER OF LARVAE AND THE PERCENTAGE NON-MOTILE WAS ESTIMATED

resuspended in 10ml of distilled water. A 0.5ml sample was made up to 10ml with distilled water and 0.2ml aliquot samples were transferred to a three-well glass dimple slide. The number of motile and non-motile larvae present in each 0.2ml of the diluted solution (1:20) was counted using a stereo-microscope. From these data the mean number of larvae present per ml of the stock suspension and the percentage of non-motile larvae - which was taken as a measure of mortality, was estimated, from six aliquots.

In experiments only freshly emerged larvae were used, but third-stage larvae stored at 19°C ± 1°C in distilled water in an anhydric incubator with an internal fan circulation of air, were often used to infect stock rats. Stock rats were infected under ether anaesthesia by subcutaneous injection of 1000 third-stage larvae in 0.3ml distilled water, using a 19G needle (Sabre, Gillette) and a 1ml Plastipak syringe (B.D.).

## 2.2. Rats

Two strains of rat were used throughout this study, random-bred *Rattus norvegicus* Wistar strain, and inbred *Rattus norvegicus* PVG strain. The rat colonies involved were constant and originally managed by the Centre for Laboratory Animals, Bush Estate, Penicuik, but from 4th. Septtember 1984 they were taken over by the Institute of Occupational Medicine which maintained them in the same premises. Three-week old female Wistar rats were used for passage and from October 1982 to the 16th October 1984 four rats were infected for each strain or species. However, from 23rd. October 1984 to the end of the study the number was reduced to two for financial reasons. The Zoology Department started its own stock breeding programme in December 1984 using Wistars from the Bush Estate as the original parents, and became

self-sufficient in regard to this strain by January 1985. From this time both male and female rats were then used for passage to avoid wastage. Animals were housed in one of twc types of cage: stock cages, (Model RB3, North Kent Plastics Ltd., Home Garden, Dartford, Kent) with white wood shavings in the bottom, or grid cages, (Model R1, North Kent Plastics Ltd.). Water, provided by an automatic watering system, and food (Diet 41B, Oxoid, manufactured by Herbert C. Styles Ltd., Bewdley) were provided *ad libitum*. The rats were kept in either of two rooms, designated M and G, at 20-22°C, with a continuous light cycle, a carryover of former migration studies. From December 11th. 1984, rats in room M were placed on a 12 hour light:12 hour dark regime to facilitate the stock rat breeding programme.

## 2.3. Production of radio-active labelled third-stage larvae and estimation of the proportion of labelled dose recovered

(After Wilson and Simpson, 1982)

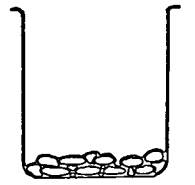
### 2.3.1. Production of radio-active labelled third-stage larvae

(Fig.2:5, page 34)

The underlying culture method was essentially the same as previously described (Section 2:1, page 30). One or two faecal pellets were placed in each well of a three-well glass dimple slide, which was placed in a Petri-dish and 0.3ml of diluted $^{75}$-Se-selenomethionine (Amersham International Ltd, Appendix 1, page 278 ) solution was added to each well using a 1ml Plastipak syringe (B.D.), and distilled water was added to the dish, before covering with its lid. After the required culture period the *Strongyloides spp.* third-stage larvae were harvested using a 5ml Plastipak syringe (B.D.) with a plastic tube attachment instead of a needle. Their number and the percentage non-motile were estimated (Section 2:1, page 30). Free radio-active label was removed by

Fig. 2:5

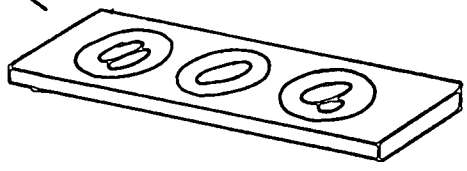
Preparation of radio-labelled third-stage larvae



FAECES WERE COLLECTED AND WASHED IN DISTILLED WATER

1 → INFECTION WAS CONFIRMED MICROSCOPICALLY

2



one or two Faecal pellets were placed in each well of a three-well dimple slide

0.3ml of $^{75}$Se-Selenomethionine was added to each well activity : 74kbq per ml

3

THE SLIDE WAS PLACED IN A PETRI-DISH WITH A SMALL AMOUNT OF DISTILLED WATER. THE DISH WAS LABELLED WITH THE STRAIN/SPECIES.

4

THE FAECES WERE INCUBATED AT THE REQUIRED TEMPERATURE UNTIL INFECTIVE LARVAE WERE PRODUCED

5

THE NUMBER OF THIRD-STAGE LARVAE PRESENT, THE PERCENTAGE NON-MOTILE AND THE NUMBER OF COUNTS PER MINUTE PER LARVA WAS FOUND

repeated washing with distilled water, until the gamma emission from a 0.5ml sample of the supernatant approached background levels using a Panax counter. Having estimated the number of larvae per unit volume of a suspension (page 30), three samples of different volume were taken to measure the mean counts per minute per larva.

### 2.3.2. Proportion of the radio-labelled dose recovered

Rats were infected by skin-application (see Section 2:10:1, page 48) of third-stage larvae in 0.3ml (approximately 2000 larvae). At the appointed time they were killed by carbon dioxide asphyxiation, their body cavities were opened using scissors, and their intestines removed with forceps. The intestines were treated in one of two ways:-

(i) separated into anterior and posterior sections and placed into glass counter vials with 5% formalin. The amount of activity per 1000sec for each section was counted using a Nuclear Enterprises gamma spectrometer (setting for 75-Se, threshold E=270KeV, window width E=170KeV). Supernatants and larval suspensions were counted at the same time to avoid discrepancies caused by decay.

(ii) divided into anterior and posterior sections and stored at -20°C until an appropriate time when the amount of activity for each section per 1000sec was estimated with the Panax counter and expressed as counts per minute (cpm). The number of adult worms in each section was then counted with a stereo-microscope (Section 2:10, page 50) to find out how well the levels of radio-activity correlated with the actual numbers of adults present. Similarly supernatants and larval suspensions were counted at the same time to correct for radio-active decay. From these data the proportion of the radio-labelled

dose recovered could be calculated, using the formula below:-

$$P = \frac{m}{y}$$

Where :-

P = proportion of the dose recovered
m = cpm per gut section (i.e. anterior or posterior)
y = cpm of the original dose used to infect rats

Background counts were subtracted from all data.

## 2.4. Preparation of heat-killed larvae to test for protective antigens

The number of third-stage larvae harvested from cultures was estimated (Section 2:1, page 30), and the stock larval suspension concentration adjusted to 10,000 infective larvae per 0.3ml. Larvae were heat-killed by placing in a boiling water-bath for two minutes, since preliminary studies had shown this was sufficient time to render all larvae non-motile (which was taken as an indication of death), and clumping did not occur. The suspension was allowed to cool before it was transferred to Eppendorf tubes, and then stored at -20°C until required. When needed the suspension was defrosted at room temperature. The freezing process did not induce clumping and re-estimation of larval numbers showed that little fluid was lost at any time, so that the concentration of the suspension remained at 10,000 third-stage larvae per 0.3ml.

## 2.5. Adoptive transfer of mesenteric lymph node cells

(after Moqbel and Wakelin, 1981)

### 2.5.1. Preparation of mesenteric lymph node cells

Rats, killed by carbon dioxide asphyxiation in batches of not more than 7 animals, had their ventral surface swabbed with 90% alcohol and the body cavity opened using clean, flamed scissors. The mesenteric lymph nodes were located and removed using fine, curved forceps, which had been flamed. Nodes from the same treatment were pooled in a small sterile beaker containing cell medium (Appendix 1 , page 278 ). This dissection was completed within ten minutes of the death of each animal. Cell suspensions were prepared from a few nodes at a time in a tissue grinder containing cell medium held in an ice pack. The nodes were carefully disrupted until the membranes were free of most cells, and the resulting cell suspension was passed through a fine sterile sieve into a sterile glass tube held in an ice pack. Cells from individual treatments were pooled.

A 20µl aliquot taken from an even suspension of each cell preparation was resuspended with 180µl of the vital stain acridine orange /ethidium bromide (Appendix 1, page 278), in an Eppendorf tube. A small sample of the stained cells (approximately 20µl) was loaded into a haemacytometer (standard counting chamber, depth 0.1mm, 1/400mm, Hawksley, Lancing, Sussex), and viewed under a fluorescence microscope (Vickers photoplan with 100 and 200 watt power units, 495nm primary filter and 515nm secondary filter) to determine the number of live (green) and dead (red) cells. By this test the viability of cell preparations used for transfer experiments always exceeded 85%.

### 2.5.2. Adoptive transfer

Each rat received 1-2.5x$10^8$ cells in 0.5ml of cell medium. The cells were injected into a tail vein of an unanaesthetised, restrained animal (Fig.2:6, page 39) using a 1ml Plastipak syringe (B.D.) and a 25G needle (Sabre, Gillette). Control animals received 0.5ml of cell medium. Prior to injection rats were placed in a "hot-box" (a plastic container heated with two 60 watt bulbs), for a few minutes, in order to bring the veins to the surface.

## 2.6. Lymphocyte transformation assay

(Fig.2:7, page 40).

A mesenteric lymph node cell suspension in cell medium (Appendix 1, page 278) was prepared (Section 2:5, page 37), the concentration was adjusted to 2x$10^6$ cells per ml and 100ul added to each well of a 96 well flat-bottomed microtitre-plate (NUNC). Cells were stimulated with 50µl of different antigen preparations at various concentrations, or concanavalin A (Appendix 1, page 279) which acted as a positive control to show that cells were capable of being stimulated. Unstimulated cells, received 50µl of serum-free medium (Appendix 1, page 279). Six replicates were set up for each treatment and an individual plate was prepared per rat. The plates were incubated at 37°C for 4 days in a carbon dioxide incubator (Forma Scientific, 5% carbon dioxide: 95% air).

A small sample was removed from one well in each treatment, immediately before the addition of tritiated thymidine (Appendix 1, page 278) to the cells. The sample was transferred to an Eppendorf tube in order to check cell viability by direct on slide centrifugation (Cytospin; Shandon Scientific) followed by staining. A one minute centrifugation with cell medium was executed to facilitate the attachment of cells to the slide surface following which the

Fig 2:6

Method of immobilising the rat for cell transfer

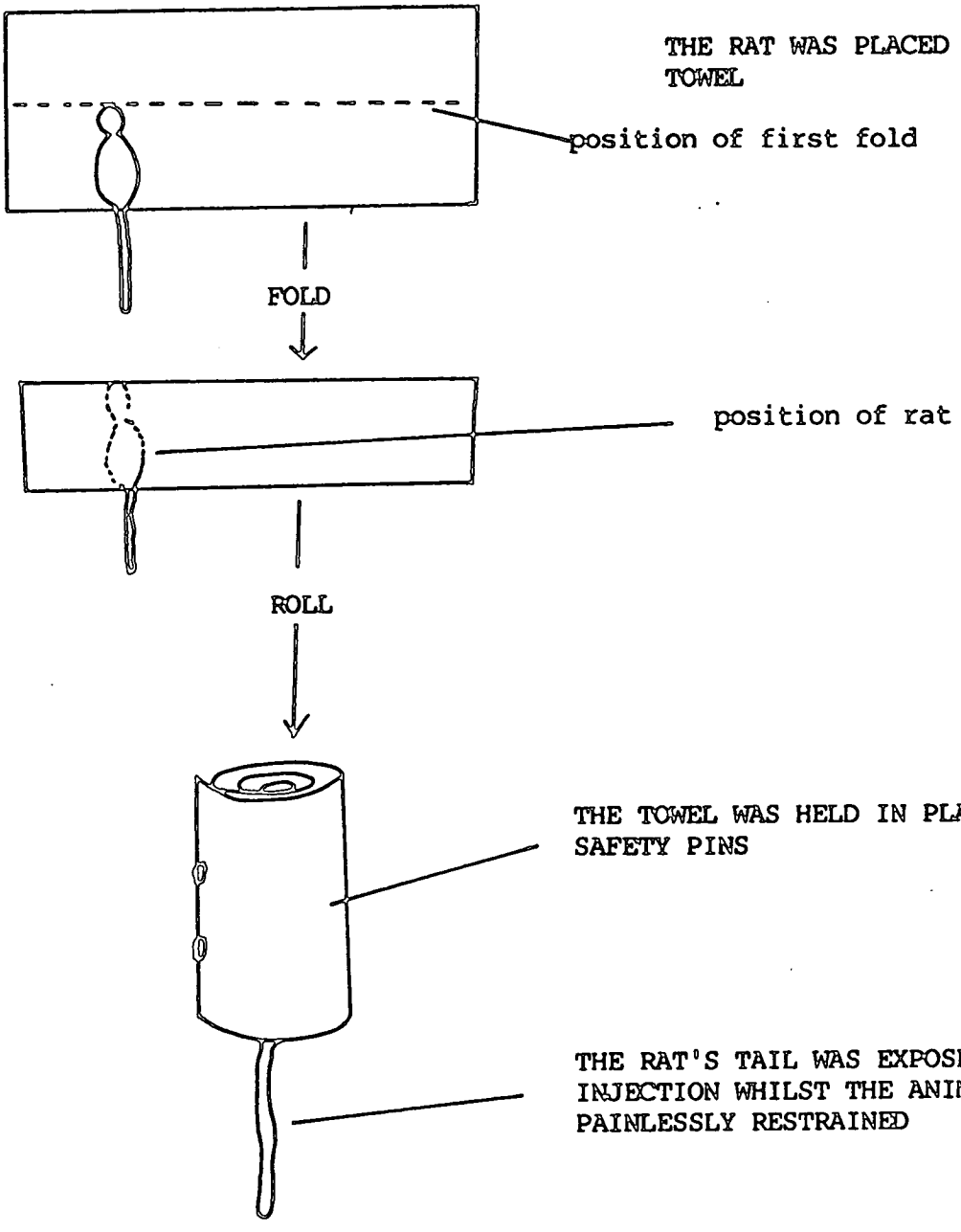


Fig. 2:7

The lymphocyte transformation assay

MESENTERIC LYMPH NODE CELLS WERE COLLECTED AND THEIR NUMBER AND VIABILITY WAS ESTIMATED. THE CELLS WERE SPUN DOWN AND THEN RESUSPENDED IN CELL MEDIUM TO GIVE A CONCENTRATION OF $2 \times 10^{6}$ CELLS PER ML

1

CELLS WERE PLATED OUT IN A 96 WELL FLAT-BOTTOMED MICROTITRE PLATE UNDER A STERILE HOOD

2

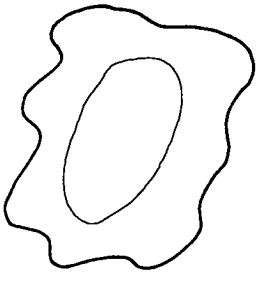
THE PLATE WAS PLACED IN A CARBON DIOXIDE INCUBATOR (5% CARBON DIOXIDE : 95% AIR) AT $37^{\circ}$C UNTIL DAY 3

3

A SMALL CELL SAMPLE WAS TAKEN FROM ONE WELL OF EACH TREATMENT FOR DIRECT ON SLIDE CENTRIFUGATION. THE CELLS ON THE SLIDE WERE STAINED AND EXAMINED MICROSCOPICALLY TO SHOW VIABILITY

4

0.5µCi (18.5kBq) OF TRITIATED THYMIDINE WAS ADDED TO EACH WELL THAT WOULD BE HARVESTED IN 20µl.



A HEALTHY CELL WITH A LARGE NUCLEUS AND INTACT CELL MEMBRANES

5

ON DAY 4 THE CELLS WERE HARVESTED ON TO FILTER PAPERS

6

THE PAPER WAS ALLOWED TO DRY PRIOR TO PLACING EACH FILTER DISC INTO A SCINTILLATION VIAL WITH SCINTILLANT

7

THE AMOUNT OF LABEL PRESENT IN THE CELLS WAS COUNTED

sampled cells were loaded into the cytospin and spun at 1500g for 4 minutes. Excess suspension was absorbed by a filter card (Shandon 450gm/mm), leaving a distinct circle of cells on each slide, which was air-dried, fixed in methanol for 30 seconds, then stained for 30 minutes in 10% Giemsa (Gurr). "Healthy" cells had distinct nuclear and cell membranes. If >40% of the cells were dead the experiment was abandoned. In experiments involving a variety of treatments (i.e. more than 5), cell samples from only 2 or 3 treatments were taken for assessment of cell viability, but different treatments were randomly chosen for each rat.

On day 3 (at approximately 4pm), 18.5kBq of [methyl-$^{3}$H]- thymidine (Appendix 1:1, page 278) in 20µl of serum-free medium was added to each well which would be harvested, before replacing the plate in the incubator. Seventeen hours later the cells were collected using a Titertek Cell Harvester, which washed them free of unincorporated tritiated-thymidine and concentrated the cells from each well on to a small disc of filter paper (Titertek, Flow Laboratories). After drying, each disc was placed into a glass scintillation vial with 5ml of scintillant (Appendix 1, page 279), or later into disposable plastic scintillation vials in 1ml of scintillant.

## 2.7. Preparation of parasite antigens

### 2.7.1. Parasite homogenate

Approximately 600-1000 mature parthenogenetic females were recovered at day 8 post-infection (Section 2:9:1, page 44), or 60-100,000 freshly emerged third-stage larvae (Section 2:1, page 30, for method of production). These were repeatedly washed in phosphate buffered saline, supplemented with

penicillin/streptomycin (Appendix 1, page 278). Each type of preparation was transferred in approximately 2ml of supplemented saline to a tissue grinder held in an ice pack. The parasites were ground until microscopical examination of a small aliquot of the suspension revealed that considerable disruption had occurred (approximately 10-15 mins). The homogenate was spun at 500g for five minutes on a bench centrifuge (MSE) to remove the larger debris, it was then spun at 3000g for 30 minutes at 4°C on a Prepspin 50 (MSE, Fisons, Ti 10x10 rotor) to remove most of the particulate material. The resulting supernatant was sterilised by passing through a 0.22μm filter (Millipore Corporation, Bedford, Massachusetts) held in a Swinnex 25mm filter holder (Millipore, S.A., 67120 Molsheim, France) and then stored at -20°C.

2.7.2. Adult worm excretory /secretory antigen

Approximately 600-1000 day 8 parthenogenetic female worms were recovered (Section 2:9:1, page 44), repeatedly washed in supplemented phosphate buffered saline and then adult culture medium (Appendix 1, page 279) The adults were resuspended in 10ml of the adult culture medium, transferred to a 50ml tissue culture flask with an angled neck (NUNC) and incubated at 37°C in a carbon dioxide incubator, with the top of the flask loose to allow for gas entry. The medium was collected and changed every week until more than 50% of the adults were dead (which usually occurred after 2-3 weeks). The collected medium was dialysed against phosphate buffered saline at 4°C for 48 hours, concentrated by 50% using polyethylene glycol (Appendix 1:4, page 279) and then sterilised by passing it through a 0.22μm filter held in a Swinnex 25mm filter holder before storing at -20°C. The antigenic preparation obtained in this way consisted of a complex mixture of material produced by adults and first-stage larvae, since eggs laid by females hatched in culture. It

may have also contained autolysis products, since the first-stage larvae produced did not develop any further, but died.

### 2.7.3. Larval excretory /secretory antigen

The method was similar to that given in "2:7:2", 60-100,000 freshly emerged third-stage larvae (produced as described in Section 2:1, page 30) were repeatedly washed in supplemented phosphate buffered saline and then resuspended in 10ml of the saline solution before transfer to a 50ml tissue culture flask with an angled neck. The larvae were cultured at 19°C ± 1°C in an anhydric incubator with internal fan circulation of air for a week or more. The supernatant containing larval products was collected and renewed every week and treated exactly as described in "2:7:2" for the adult antigen.

### 2.8. Protein determination using a micro-Lowry technique

(After Lowry *et al*, 1951) One millilitre of ice-cold trichloracetic acid (Appendix 1:5, page 281) was added to 100µl of the test sample before vortexing for 15 minutes (Whirlimix, Fisons). The mixture was then spun at 13,000g for 2 minutes on a micro-centaur (MSE), and the resulting supernatant was aspirated off and discarded. One hundred microlitres of 1M sodium hyroxide solution (Appendix 1:5, page 281) was added to the pellet, the mixture was vortexed and then incubated for ten minutes at room temperature. One millilitre of solution D (Appendix 1:5, page 281 ) was added with mixing, and then the solution was incubated for a further 10 minutes before 100µl of solution E (Appendix 1:5, page 281) was added with mixing. This final solution was incubated for another 30 minutes and then the absorbance of the samples at 750nm was read using a SP8 4000 UV/VIS spectrometer (Pye, Unicam). The protein concentration of the test samples (two replicates for each sample) was

found from a standard curve plotted from protein standards (BSA, Sigma), ranging from 0.125-1mg protein per ml, run at the same time.

## 2.9. Surgical transfer of adult parthenogenetic female worms from one rat to another

### 2.9.1. Recovery of adult worms

Mature female worms were recovered on day 8 post-infection from four three-week old Wistar stock rats infected by subcutaneous injection under ether anaesthesia with 2000 third-stage larvae. Rats had been starved since day 7 post-infection (p.m.) in order to minimise the amount of food present in their intestines.

Rats were killed by carbon dioxide asphyxiation, their ventral surface swabbed with 70% alcohol and the body cavity opened using flamed scissors. The stomach and 20cm of the attached small intestine was removed, using flamed scissors and large forceps, to a Petri-dish containing supplemented phosphate buffered saline at 37°C. The stomach was removed and the intestine opened longitudinally using small scissors and fine forceps. Large intestinal debris was carefully scraped away and fresh saline added to cover the intestine, which was then incubated for 80 minutes at 37°C, by which time the adults had migrated into the saline. This process was usually completed within 10-15 minutes of the death of the rats.

### 2.9.2. Adult transfer

Adult parasites from a particular rat were collected and a maximum of 6 doses of 50 adults, in approximately 0.3ml saline, were individually

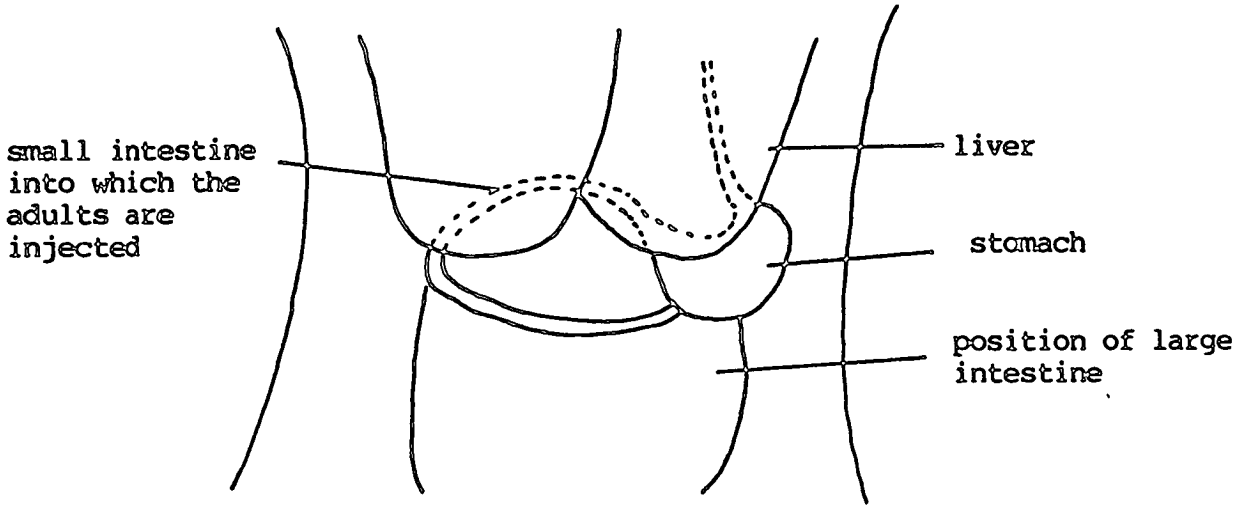
hand-counted into a solid watch-glass. Rats which were to receive adults recovered from one animal were anaesthetised with "Sagatal"(0-3mg per 100gm body weight, pentobarbitone sodium, May and Baker) and their abdominal and chest fur removed using "Immac$^{R}$"cream (Anne French, London). The ventral surface of the rat was swabbed with 70% alcohol, before making a longitudinal incision of approximately 2cm into the body cavity behind and slightly to the left of the sternum using a sterile scalpel (No. 3, Paragon; with a No.10 blade, Swann Morton Ltd.,Sheffield). The intestine was exposed by lifting one of the cut edges of the tissue layer with sterile forceps. The pyloric end of the small intestine was raised from behind the liver, through the incision with fine forceps. Holding it, now, with large forceps, the loop of the small intestine was secured in its external position by passing the fine forceps through it, so as to form a bridge across the incision in the body wall (Fig.2:8, page 46). The dose of 50 adults was injected into the intestine, using a 1ml Plastipak syringe and a 19G needle , before allowing it to return to the body cavity by removing the restraining fine forceps. A small amount of Terramycin$^{R}$powder (<5mg, oxytetracycline, Pfizer) was placed inside the body cavity before the muscle layer of the peritoneum was sewn up using a 1/2 inch spring-eyed, round-bodied, surgical needle (No.20, Surgicraft, A.W. Showell Ltd., Redditch) threaded with black braid non-capillary suture silk (Suture Ltd., Dyffryn Estate, Newtown, Powys). The skin layers were autoclipped together using 12mm autoclips (Michel, Germany). The incision area was sprayed with Alamycin$^{R}$ (oxytetracycline hydrochloride B.P. Gentian Violet, Norbrook Laboratories Ltd., Newry, Northern Ireland), before replacing the rat in its cage. All instruments were swabbed with absolute alcohol between transfers. The surgical needle and autoclips were stored in the same liquid.

Rats were maintained on Terramycin$^{R}$ (165mg per litre) in their drinking

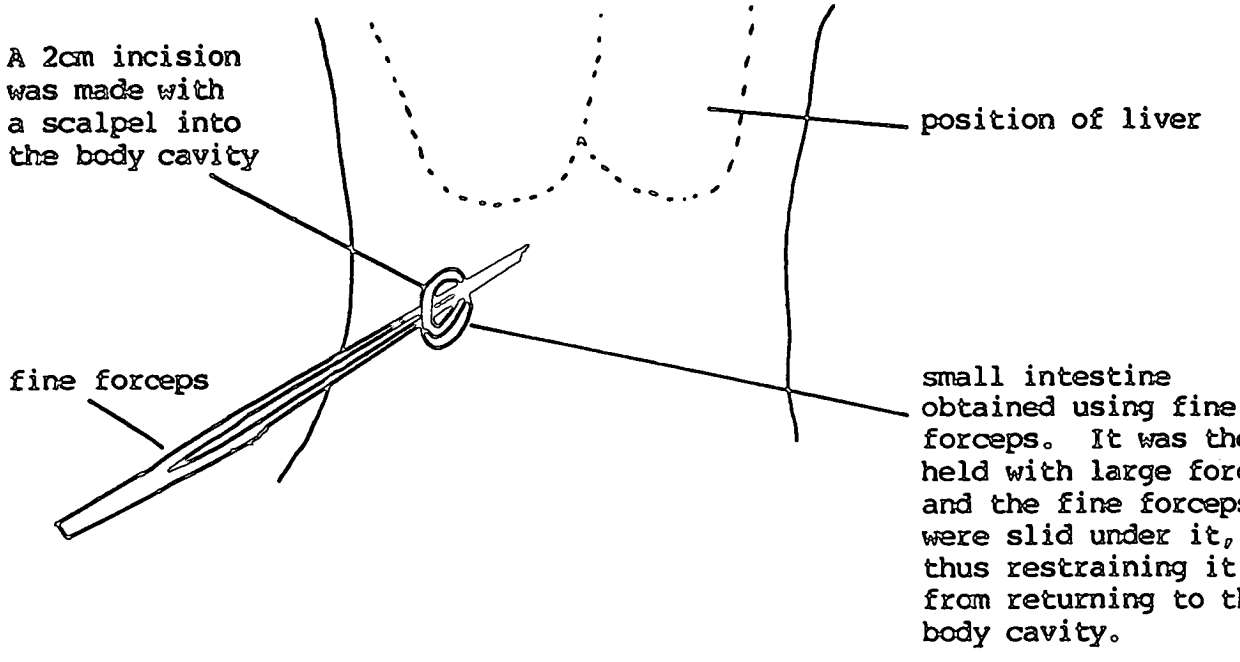
Fig. 2:8

The method used to obtain the required section of small intestine for the surgical transfer of adult worms

a) The relative position of the rat's internal organs




b) The site of the incision into the rat's body cavity and the method of restraining the small intestine in its external position



water for 5 days post-infection. On day 4 post-infection autoclips were removed whilst the animals were held by an assistant. The exact number of adults injected into each rat was found by recovering those that remained in the watch-glass and syringe with its attached needle.

thus:

$$D = 50 - [a + b]$$

Where:-

D = exact dose for a particular rat

a = number of adults left in the solid watch-glass for the same rat

b = number of adults recovered in washings from syringe and its attached needle for the same rat

Donors were killed as worms from the proceeding animal were used up. This ensured that recipients were given parasites exposed to the outside environment for a minimum time of approximately 80 minutes.

## 2.10. Infection procedure used in experiments

Newly emerged third-stage larvae were collected from faecal cultures (Section 2:1, page 30), pooled, and then doses of 100 larvae in approximately 0.3ml of distilled water were individually hand-counted into a solid watch-glasss from one of three Petri-dishes containing the larval suspension. The dishes were rotated to ensure that the time any one was exposed to the sub-stage illumination was less than 4 minutes, thus attempting to avoid the possibility that heat from the stage of the microscope might impair larval viability. The temperature of the stage was approximately 20°C. Rats were infected in one of four ways:-

### 2.10.1. Skin-application, after Wilson *et al*, 1984

Rats were immobilised for two-three hours under "Sagatal" anaesthesia (0.3mg per 100gm of body weight, pentobarbitone sodium, May and Baker), given one hour prior to infection. The interim period of one hour is required to eliminate the effects of the anaesthetic on the infection process which otherwise occur (Wilson, Simpson and Seaton, 1986). During this hour a 0.5cm recess was clipped into the fur on the right flank of each rat, to expose the skin without breaking its surface, using flat-bladed scissors. The larval dose was applied to the wetted recess in the rat's fur using a 1ml Plastipak syringe, whilst it laid in its cage. The rats were then left undisturbed until they recovered consciousness.

### 2.10.2. Injection under ether, after Wilson and Simpson, 1981

Rats were injected subcutaneously in the right flank with the larval dose using a 1ml Plastipak syringe and a 19G needle, whilst under ether anaesthesia. A second injection of 0.3ml of distilled water was given in the same area with the same syringe, in an attempt to flush out any larvae trapped in the needle. Animals were returned to their cages and allowed to recover. Wilson and Simpson (1981) have shown that ether anaesthesia administered in this way does not affect worm development.

### 2.10.3. Injection, no anaesthesia

"2:10:2"

The same procedure described in was followed, rats were injected whilst held by an assistant.

### 2.10.4. Infection with a large estimated dose

In some experiments rats were infected with a large dose of larvae, e.g. 2000 third-stage larvae. In this case the total number of larvae recovered from fresh cultures was estimated (Section 2:1, page 30) and the concentration of the stock larval suspension adjusted to give the required mean number of larvae per 0.3ml.

After injection or skin-application of hand-counted doses the exact number of larvae given to each rat was determined as precisely as possible by counting the number of residual larvae. The number of larvae left in each watch-glass was obtained by adding a small drop of distilled water to it (approximately 0.2ml), and then scanning with a stereo-microscope. Larvae trapped in the syringe or syringe with its attached needle were dislodged by repeated flushing with distilled water into a glass centrifuge tube. The larvae were concentrated by centrifuging at 500g for 3-4 minutes on a bench centrifuge (MSE), the supernatant was aspirated off and discarded, and the larvae were transferred to a solid watch-glass for counting under a stereo-microscope.

Thus:

$$D = 100 - [a + b]$$

Where:-

D = exact dose for a particular rat
a = number of infective larvae present in solid watch-glass after infection for the same rat
b = number of larvae recovered from the washings of the syringe or syringe and its attached needle for the same rat

On the required day post-infection (usually day 8) the rats starved from the proceeding afternoon, were killed by carbon dioxide asphyxiation, the body cavity was opened with scissors and the intestine, including the stomach and caecum, was removed and stored in a sealable plastic bag at -20°C. At a convenient time a gut was defrosted under a bench lamp, the stomach removed, and the intestine was divided into 8 sections from the pylorus to the beginning of the caecum. Each intestinal segment was opened longitudinally and large debris removed with fine forceps, before squashing it between two glass slides (approximately 14x14cm$^2$). Four sections were loaded per plate and the number of adults present in each section was counted using a stereo-microscope (x40 magnification). To facilitate counting the top slide had a 12x12cm$^2$ grid of 144 squares etched on its surface. The same preparations were used to estimate the mean number of eggs in utero per worm for each rat by counting this feature in 20 randomly chosen worms. In order to prevent rats carrying a worm burden from re-infecting themselves from their own faeces, their cage bottoms were replaced every two days from day 7 post-infection. Worm burdens were expressed as a proportion of the exact dose given to each rat and the results were analysed as shown in Section 2:11

(page 51).

Within each experiment there was a group of animals (commonly referred to as "cosmic controls") infected with an exact dose of <100 third-stage larvae of the relevant parasite, which were killed on day 8 post-infection. These were used to assess the viability of the larvae used at priming since comparison of the proportion of the dose recovered from these animals with past data collected in this laboratory indicated whether there was a possibility that the larvae used were defective. Initially these animals were infected by subcutaneous injection without anaesthesia since this was the mode of infection used in the past in this laboratory, but in later studies rats were infected using the skin-application technique (see section 2:10:1, page 48) since the majority of the animals were infected in this manner and by this time enough data had been collected to use as reference.

## 2.11. Statistical analysis of results

### 2.11.1. Worm data

(after Wilson and Simpson, 1981)

Worm burdens were expressed as a proportion of the exact dose given to an individual rat. Thus:-

$$P = \frac{N}{D}$$

Where:-

P = proportion of the dose recovered from a particular rat
N = total number of parasites recovered from the same rat
D = exact dose given to the same rat

The mean proportion of the dose recovered for a particular treatment was calculated as shown below:-

$$P = \frac{\Sigma n_p}{n}$$

Where:-
P = mean proportion of the dose recovered for a treatment
n = number of rats within the treatment

Treatments within experiments were compared using a one-way or a two-way analysis of variance on the arc sines of the data. Least significant differences between means were estimated from the pooled error variance in the standard way (see Sokal and Rohlf, 1969, pages 240-246)

2.11.2. Egg data

Preliminary studies showed that the egg data, although discontinuous, had a distribution which was not significantly different from normal (Chapter 3, Section 3:8, page 77). The mean number of eggs *in utero* per worm for different treatments (groups) within an experiment were compared using a two-level nested analysis of variance. If there was no significant difference between rats within groups but a significant difference between groups, then the least significant difference between means was calculated (as given in "2:11:1") from the pooled error variance, using the mean number of worms per rat as the divisor to calculate the pooled standard error. The "t" value of the degrees of freedom related to the error variance was used in calculations. Ideally the subgroup (=rat) and the group mean squares and the subgroup degrees of freedom should be corrected for unequal sample sizes by a

somewhat complicated procedure (Sokal and Rohlf, 1969, pages 274-281); however experience showed that such corrections had little quantitative effect and led to no changes in terms of the significance of differences between group means (Appendix 1, shows an example, page 282). These corrections therefore were not routinely made.

If results of the two-level nested analysis of variance showed there was a significant difference between subgroups (=rats) within groups, the subgroup means were used as single observations in a simple single classification analysis of variance. Least significant differences between group means were then calculated in the standard way (Section "2:11:1", page 51) using a "t" value with the degrees of freedom related to the number of rats per group.

### 2.11.3. Lymphocyte transformation data

Differential counts were calculated for each replicate per treatment, where appropriate, as follows:-

$$D.C. = E - U$$

Where:-

| | | |
|---|---|---|
| D.C. | = | differential count |
| E | = | the cpm for a particular treatment (e.g. cells stimulated with mitogen) |
| U | = | the mean cpm for the unstimulated control of the same rat |

The differential counts were $\log_e$ transformed (without the usual addition of one, since there were no zero values), because the standard deviations of the raw data were proportional to the means. Once transformed in this way the data fitted a normal distribution (Chapter 3, Section 3:8, page 76), allowing the application of parametric statistics. If a negative differential count was

obtained, then the negative value of the $\log_e$ was used in calculations. Thus:-

$$-D.C. = ( - [\log_e D.C.] )$$

Where:-

D.C. = differential count

Transformed data were analysed by a two-way, one-way, or a nested analysis of variance depending on the particular protocol involved (examples are shown in Appendix 8:6, pages 359-364).

Differential counts were not corrected for background since throughout experiments the background remained fairly constant at 16 cpm (Appendix 1:6, page 281). Also, the method of analysis meant that background was automatically subtracted from most results during the calculation of differential counts. To simplify the analyses of variance,equal sample sizes were used throughout. Where, occasionally, numbers of samples successfully assayed per treatment were not equal, results from individual replicates were omitted on a random basis to create a symmetrical pattern of degrees of freedom. The minimum number of replicates following this procedure was 4, though the usual number was 5, in any one treatment.

Unstimulated background controls are represented as mean $\log_e$ counts per minute.

### 2.11.4. Quantitative comparison of homologous and heterologous challenge results

In order to compare the relative reduction in the number of eggs *in utero* per worm, or the relative reduction in the proportion of the dose recovered, after homologous and heterologous challenge, it was necessary to calculate "resistance ratios", which took into account differences in the control values for individual treatments. Where:-

#### 2.11.4.1. Worm data

$$Rw = \frac{Pe}{Pc'}$$

$$Pc' = \frac{\Sigma^{n} Pc}{n}$$

Where:-

| | | |
|---|---|---|
| Rw | = | resistance ratio for the worm data (homologous or heterologous challenge group) |
| Pe | = | proportion of the dose recovered from either homologous or heterologous challenged rats (i.e. one observation) |
| Pc | = | proportion of the dose recovered from corresponding controls (i.e. one observation) |
| $Pc'$ | = | mean proportion of the dose recovered from corresponding controls |
| n | = | number of rats |

2.11.4.2. Egg data

$$Re = \frac{Ute}{Utc'}$$

$$Utc' = \frac{\Sigma^{n} Utc}{n}$$

Where:-

| | | |
|---|---|---|
| Re | = | resistance ratio for egg data (homologous or heterologous challenge treatment |
| Ute | = | mean number of eggs *in utero* per worm per rat of homologous or heterologous challenged rats (i.e. one observation) |
| Utc | = | mean number of eggs *in utero* per worm per rat of corresponding controls (i.e. one observation) |
| Utc' | = | mean of means of corresponding control eggs *in utero* per worm per rat |
| n | = | number of rats |

The resistance ratios for different treatments were then analysed in the same way as described in "2:11:1" for the worm data (page 51), and in "2:11:2b" for the egg data (page 52), treating each resistance ratio as a single replicate within its corresponding treatment. Least significant differences between group means were then calculated, using the pooled error variance, with its related degrees of freedom (see Sokal and Rohlf, 1969, page 240-246). For simplicity these resistance ratios were diagrammatically expressed as a "resistance quotient", calculated as shown below:-

$$ReQ = 1 - ( R )$$

Where:-

| | | |
|---|---|---|
| ReQ | = | resistance quotient |
| R | = | mean resistance ratio for a particular treatment (either the homologous or heterologous challenge group) |

Thus the height of a bar in the relevant histograms is related to the amount of protection expressed. The higher the bar, the greater the immunity demonstrated. The "P" value calculated using the resistance ratios are shown on these diagrams since the mean resistance ratio per treatment and the mean resistance quotient for the same treatment, have the same standard error and standard deviation (Appendix 1:8, page 288, shows a worked example of how the resistance ratios and resistance quotients were calculated and their relationship).

2.11.4.3. Presentation of data

Throughout this study the results of a particular experiment are either shown in a figure, in a table, or quoted directly (where the standard error is given along with the mean of a particular treatment).

# CHAPTER 3

# TESTS OF METHOD

## 3.1. Introduction

This chapter includes a number of experiments which were carried out for one of two reasons:-

either a) to show that some alteration in the experimental protocol did not affect the result obtained

or b) to develop a new experimental system For simplicity the chapter is divided into sections, each of which contains an integrated description of experiments on a common theme.

## 3.2. Section 3:1

### 3.2.1. Pairing of rats [Expt 1]

Since experiments usually covered a period of 4-6 weeks, individual experiments overlapped in time. There was insufficient cage space in the animal house to accommodate the large number of rats involved, one to a cage, therefore it was necessary to house them in pairs. The interactions between the paired animals could include potentially stressful relationships with concommitant increases in blood corticosteroids. Bailenger and Cabannes (1976) have shown endocrine changes of this type may have an antagonistic effect on the non-specific inflammatory response, possibly leading to higher numbers of intestinal parasites. This experiment was carried

out to discover if this method of housing could have an effect on the proportion of the dose recovered. Rats were not killed until day 20 post-infection so that any difference in the worm burden of the two groups would be emphaised.

### 3.2.1.1. Materials and methods

36 rats were divided into two groups:-

Gp.A - 30 rats - experimentals, killed day 20 post-infection

Gp.B - 6 rats - controls, killed day 8 post-infection

All rats were infected with an exact dose of under 100 third-stage homogonic strain *S.ratti* larvae either by skin-application (Gp.A), or subcutaneous injection (Gp.B, "cosmic controls"). On day 1 post-infection 16 animals from Gp. A were paired, the remainder were housed singly in cages. Animals in Gp.B were killed on day 8 post-infection and were used to assess the viability of the larvae used at infection (page 51).

### 3.2.1.2. Results

Housing rats singly or in pairs had no effect since there was obviously no difference in the proportion of the dose recovered from the two treatments (mean 0.247 ± 0.031 single; 0.223 ± 0.039 paired).

## 3.3. Section 3:2

### 3.3.1. "Self-Infection" [Expt 2]

Throughout the study, experimental rats carrying a worm burden were maintained in the same cage for up to six weeks. Therefore there was a possibility that these animals could become re-infected by the skin penetrating third-stage larvae, which had developed from their own faeces; so it was nessary to find out whether this actually happened, and if so, whether a strict cleaning regime prevented its occurrence.

#### 3.3.1.1. Materials and methods

34 rats were divided into three main groups:-

Gp. C - 6 rats - controls, killed day 8 post-infection

Gp. I - 14 rats - infected, killed day 20 post-infection

Gp. U - 14 rats - uninfected, killed day 20 post-infection

All animals were kept in cages with closed plastic bottoms containing wood shavings. Members of Gps. I and C were infected with an exact dose of below 100 third-stage homogonic *S.ratti* larvae either by subcutaneous injection (Gp.C, "cosmic controls" used to estimate viability of larvae used to prime rats, page 51) or skin-application (Gp.I). On the day after infection animals were paired by placing infected rats (Gp.I) into the cages already occupied by the uninfected ones (Gp.U). In the case of 7 of the pairs, cage bottoms were renewed on day 8 and every 2 days subsequently. The cage bottoms of the 7 remaining pairs were renewed only once, on day 10

post-infection.

3.3.1.2. Results

Microscopical examination of faeces collected from infected rats showed that eggs were not present until day 6 post-infection therefore third-stage larvae, capable of infecting animals, would have been produced by day 9 post-infection. However none of the uninfected rats became infected suggesting that rats are unable to re-infect themselves from their own contaminated faeces. In confirmation, there was no significant difference in the parasite burden of infected rats exposed to either cleaning regime (mean proportion of the dose recovered 0.340 ± 0.045 changed every two days; 0.403 ± 0.067 changed once).

3.4. Section 3:3

Surgical transfer of adult worms between rats [Expts 3, 4 and 5]

A new experimental system had to be developed to carry out surgical transfer of adult worms, in which various factors that could adversely affect the survival of parasites were explored and eliminated. Additionally, practical information on the donor:recipient ratio had to be obtained.

3.4.1. Expt 3, Time course of emergence of adult parasites from the intestines of freshly killed rats

3.4.1.1. Materials and methods

Two, three-week old, female Wistar stock rats infected with 1000 infective larvae of homogonic *S.ratti*, heterogonic *S.ratti*, or *S.venezuelensis* were killed

on day 7 post-infection. The body cavity of each rat was opened using scissors and the first 20cm of their small intestine was removed to a Petri-dish containing warm saline in an incubator at 37°C; a process completed within 5 minutes of the donor's death. The number of adults which had migrated out into the saline was counted at 30 minute intervals, starting with the time when the first ones appeared.

### 3.4.1.2. Results

The majority of parasites were recovered between 90-120 minutes of incubation and appeared to be very active, in contrast those gathered after four hours, were sluggish or dead, and obviously unsuitable for transfer. In both strains of *S.ratti* and *S.venezuelensis* 200 or more adults were collected in the 90-120 minute period (Table 2:3(i), Appendix 2, page 291) suggesting that a 1:4 or 1:5 donor to recipient ratio would be feasible if rats were to be infected with 50 adult worms.

## 3.4.2. Expt 4, The effect of time spent in culture on worm survival

### 3.4.2.1. Materials and methods

Four groups of four rats (Gps.A, B, C and D) were infected by the surgical transfer technique (Section 2:9, Chapter 2, page 44) with an exact dose of less than fifty adult homogonic *S.ratti* worms (mean 49.10). All doses were prepared from the pooled worm burdens of 4 donor rats killed at the same time. Parasites were taken from this pool at 1.5h, 2.5h, 3.5h and 4.5h to infect rats in Gps.A, B, C and D respectively. Animals were killed on day 4 post-infection.

3.4.2.2. Results

There was a significant reduction in the proportion of the dose recovered if rats were infected with doses which had been prepared more than one and a half hours after the donor's death (Table 2:3(iii), Appendix 2, page 291). However, there was no significant difference in the mean number of eggs *in utero* per worm for the four treatments. Therefore the results suggested that to ensure maximum survival adult worms should be transferred within two hours of the donor's death.

3.4.3. Expt 5, Viability of transplanted worms from different donors

The results of Expt 4 had shown that parasitic female worms used for transfer should only be exposed to the external environment for up to 2 hours, since those transferred any later than this have a reduced ability to establish. It was only possible to infect a maximum of six rats within 30 minutes and sufficient adults for transfer only emerged from culture after 90 minutes. Therefore it would be necessary to stagger the death of the donors to ensure that adults were transplanted within this time limit. Thus it was essential to discover if females obtained from different donors had equal abilities to establish in a new host.

3.4.3.1. Materials and methods

Four batches of four rats were infected by laparotomy (Section 2:9, Chapter 2, page 44) with an exact dose of <50 adult *S.venezuelensis* worms prepared from one of four donors, the time of whose deaths had been staggered by hourly intervals to ensure that the transferred worms had only been exposed

to the external environment for a maximum of two hours. Animals with surgically transferred worms were killed four days later.

#### 3.4.3.2. Results

There was no significant difference in the proportion of the dose recovered from animals which received worms from different donors (overall mean 0.281 ± 0.043), suggesting that varying the donor had no effect on the ability of transplanted worms to establish in a new host.

## 3.5. Section 3:4

### 3.5.1. "Autoinfection" [Expt 6]

Although never proven, the process of "autoinfection" is still implicated by some authors in the biology of *Strongyloides spp.* including *S.ratti* (Moqbel and Denham, 1978). According to their idea, first-stage larvae produced by adults in the intestine need not pass to the outside of the host, but may "metamorphose" into infective larvae endogenously and thus add to an existing worm burden. All the experimental results were explicable without recourse to the idea of autoinfection, but the surgical transfer technique was employed, using first-stage larvae instead of adult worms, in an attempt to test the hypothesis.

#### 3.5.1.1. Materials and methods

First-stage homogonic *S.ratti* larvae were obtained by passing the supernatant, collected from culturing the small intestines of four stock rats in supplemented phosphate buffered saline at 37°C (Section 2:9:1, Chapter 2,

page 44), through double thickness cotton gauze. This procedure removed intestinal debris and adult worms leaving a solution containing only first-stage larvae. The larvae were washed in saline, their number estimated, and the suspension was adjusted to give 450 larvae per 0.5ml. Eight rats were infected by laporatomy (Section 2:9:2, Chapter 2, page 44) with 450 larvae using a 25G needle and a 1ml Plastipak syringe and killed eight days later.

3.5.1.2. Results

Using this experimental technique no adults were recovered from any rat suggesting that auto-infection does not occur under these conditions.

3.6. Section 3:5

Cell transfer [Expts 7 and 8]

It was necessary to ensure that any reduction in the worm burden obtained after transfer of immune mesenteric lymph node cells could not be attributed to an adverse effect of the cell-transfer medium on the parasites. Therefore the effect of administering cell medium was investigated to find out if it had a detrimental effect on the establishment and survival of *S.ratti*. In the cell transfer experiments the protocol of Moqbel and Wakelin (1981) who primed rats with $2.5x10^8$ mesenteric lymph node cells was followed, therefore it was necessary to ascertain how many experimental animals could be supplied with this dose of cells from one donor.

### 3.6.1. Expt 7, The effect of cell transfer medium

#### 3.6.1.1. Materials and methods

Twelve rats were infected by skin-application with an exact dose of <100 third-stage homogonic *S.ratti* larvae (mean 98.25). Approximately 4 hours later, after the animals had recovered from the effects of the anaesthetic, half the rats were injected with 0.5ml of the cell transfer medium in a lateral tail vein (Section 2:5:2, Chapter 2, page 38). The rats were killed 8 days later.

#### 3.6.1.2. Results

There was obviously no difference in the proportion of the dose recovered from the two groups (mean 0.541 ± 0.035 no medium; 0.542 ± 0.046 medium) or in the number of eggs *in utero* per worm for the two treatments (mean 7.229 ± 0.125 no medium; 7.129 ± 0.137 medium). Therefore any reduction in the proportion of the dose recovered from animals which receive immune cells cannot be attributed to any effect of the medium.

### 3.6.2. Expt 8, Donor:recipient ratio

#### 3.6.2.1. Materials and methods

Cell suspensions of mesenteric lymph node cells were individually prepared from three uninfected rats (Section 2:5:1, Chapter 2, page 37) and the number of cells present in each and the percentage dead found.

### 3.6.2.2. Results

One rat was capable of providing up to $1.11 \times 10^9$ cells (mean $1.44 \times 10^8$) therefore a donor:recipient ratio of 1:3 or 1:4 should be possible if rats are to receive $2.5 \times 10^8$ cells each.

## 3.7. Section 3:6

Investigation into factors involved in the lymphocyte transformation assay (Expts 9, 10, 11 and 12)

A series of experiments were carried out to discover the optimum culture conditions for mesenteric lymph node cells from the inbred PVG rats used. The effect that using different concentrations of concanavalin A and harvesting cells at different times, had on the level of blastogenesis obtained *in vitro* was also investigated since a system which gave a high level of incorporation of tritiated-thymidine was required. Initially the followed the procedure of Genta *et al.* (1983), but various parameters in their protocol were altered to produce the optimum conditions for use.

### 3.7.1. Expt 9, The effect of serum concentration in the cell medium

#### 3.7.1.1. Materials and methods

Suspensions of mesenteric lymph node cells were prepared from three uninfected rats (Section 2:5:1, Chapter 2, page 37) using cell medium containing 5% human serum. The numbers of live and dead cells were estimated for each suspension. Aliquots of the original cell suspension from each rat were spun down and made up to 10ml with cell medium containing

5%, 10% or 15% human serum to give a final concentration of $2x10^6$ cells per ml. Cultures of the lymphocytes were prepared in multiwell plates with a separate plate for each rat (Section 2:6, Chapter 2, page 38) and either serum-free medium or concanavalin A (2.5μg/ml) was added to the cells (Fig.3:1, page 69). Four days later they were harvested.

### 3.7.1.2. Results

There was no significant difference in the level of unstimulated activity of cells incubated in medium containing 5 or 10% human serum. However incubation of cells in medium containing 15% human serum caused a significant reduction in the level of unstimulated blastogenesis ($P<0.001$, Fig.3:3b, page 71) suggesting that the higher serum concentration was suppressing the spontaneous activity of the cells. Each rat varied significantly in its response to mitogenic stimulation at each serum concentration (Fig.3:3a, page 71) but cells incubated in medium containing 15% human serum expressed a consistantly higher activity compared to that obtained at the lower serum concentrations.

## 3.7.2. Expt 10, The effect of varying the concanavalin A concentration

### 3.7.2.1. Materials and methods

Suspensions of mesenteric lymph node cells were prepared from three uninfected rats (Section 2:5:1, Chapter 2, page 37) and their concentration was adjusted to give $2x10^6$ cells/ml. Aliquots of the cell suspension from each rat were placed in the wells of a microtitre plate (Fig.3:2, page 69) and either serum-free medium (unstimulated control treatment) or concanavalin A, at concentrations 2.5, 5, 10 or 20μg/ml (experimentals) was added. On day 4 of

Fig.3:1

Cells from all three infected rats were cultured on the same plate. Only one plate was set up in Expt 9, but in Expts 11 and 12 three plates were prepared. Either 50µl of serum-free medium (unstimulated control treatment) or the mitogen, concanavalin A, was added to the cell suspension (100µl) contained in each well.

Fig.3:2

Aliquots of cells (100µl) from all three infected rats were dispensed into separate microtitre plates and either 50µl of serum-free medium (unstimulated cells) or concanavalin A (2.5, 5, 10, or 20µg/ml) was added. On day 4 of culture the cells were harvested and their response to the various treatments assessed.

Fig.3:1
Appearance of microtitre plate in Expts 9, 11 and 12

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | X1 | X2 | X2 | Y1 | Y2 | Y2 | Z1 | Z2 | Z2 | m | m |
| | C | m | X1 | X2 | X2 | Y1 | Y2 | Y2 | Z1 | Z2 | Z2 | m | m |
| | D | m | X1 | X2 | X2 | Y1 | Y2 | Y2 | Z1 | Z2 | Z2 | m | m |
| | E | m | X1 | X2 | X2 | Y1 | Y2 | Y2 | Z1 | Z2 | Z2 | m | m |
| | F | m | X1 | X2 | X2 | Y1 | Y2 | Y2 | Z1 | Z2 | Z2 | m | m |
| | G | m | X1 | X2 | X2 | Y1 | Y2 | Y2 | Z1 | Z2 | Z2 | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |
| | | <--cells from rat 1 --> | | | | <-cells from rat 2 > | | | <-- cells from rat 3 --> | | | | |

Where:-

m = medium only added to the cells which are not harvested
1 = unstimulated cells (same as cells with medium added but the cells were harvested)
2 = cells stimulated with concanavalin A (2.5µg per ml)
X = cells in 5% human serum (Expt 9) or cells of rat 1 (Expts 11 and 12)
Y = cells in 10% human serum (Expt 9) or cells of rat 2 (Expts 11 and 12)
Z = cells in 15% human serum (Expt 9) or cells of rat 3 (Expts 11 and 12)

Fig.3:2
Appearance of microtitre plate in Expt 10

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | U | 20 | 20 | 10 | 10 | 5 | 5 | 2.5 | 2.5 | m |
| | C | m | U | U | 20 | 20 | 10 | 10 | 5 | 5 | 2.5 | 2.5 | m |
| | D | m | U | U | 20 | 20 | 10 | 10 | 5 | 5 | 2.5 | 2.5 | m |
| | E | m | U | U | 20 | 20 | 10 | 10 | 5 | 5 | 2.5 | 2.5 | m |
| | F | m | U | U | 20 | 20 | 10 | 10 | 5 | 5 | 2.5 | 2.5 | m |
| | G | m | U | U | 20 | 20 | 10 | 10 | 5 | 5 | 2.5 | 2.5 | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |
| | | | | | <--- concentration of concanavalin A ---> in µg/ml | | | | | | | | |

Where:-

m = medium only added to the cells which are not harvested
U = unstimulated control treatment (same treatment as cells with medium but cells were harvested)

A plate was set up for each individual rat.

culture the cells were harvested and their response to the various treatments assessed (Section 2:6, Chapter 2, page 38).

### 3.7.2.2. Results

The highest level of stimulation was produced using concanavalin A at a concentration of 2.5μg/ml (Fig.3:4, page 73).

## 3.7.3. Expts 11 and 12, The effect of harvesting cells on different days

### 3.7.3.1. Materials and methods

Mesenteric lymph node cell suspensions were prepared from three uninfected rats (Section 2:5:1, Chapter 2, page 37) and their concentration was adjusted to give $2x10^6$ cells/ml. The cells from each rat were plated out (Fig.3:1 page 69) and either serum-free medium (unstimulated controls) or concanavalin A (2.5μg/ml) was added. Three plates were prepared, one of which was harvested on each of days 3, 4 and 5 of culture. Tritiated-thymidine was added to the wells seventeen hours prior to harvesting. The response of the cells to the two treatments was assessed as described in Section 2:6, Chapter 2, page 38).

### 3.7.3.2. Results

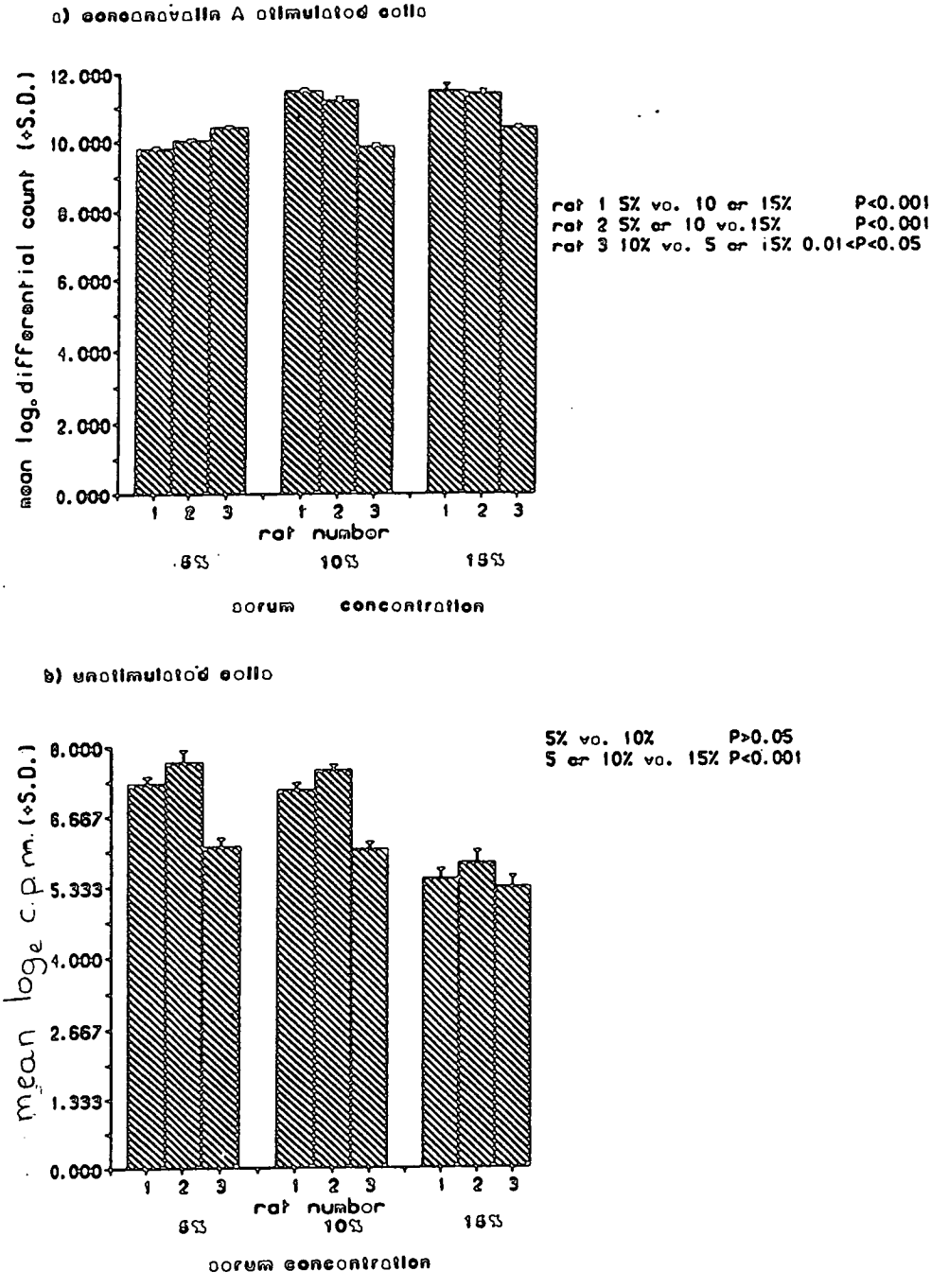
In both experiments the highest level of mitogenic stimulation was produced when cells were collected on day 3 of culture (Figs.3:5a and 3:6a, pages 74 and 75). If collected later, the level of stimulation was lower. In Expt 12 blastogenesis was reduced by 30-40% of its day 3 value on day 4, and the level of stimulation was reduced by 62-68% of its day 4 value on day 5.

Fig.3:3

Mesenteric lymph node cells obtained from three uninfected animals were suspended in culture medium containing 5, 10 or 15% human serum. The cells were cultured *in vitro* with either serum-free medium (unstimulated controls, Fig.3:3a)or concanavalin A (Fig.3:3b)to find out what effect altering the serum concentration of the cell medium had on the level of stimulation obtained.

Fig.3:3

Effect of varying serum concentration in the medium, Expt 9



Unfortunately there are no results for day 5 from Expt 10 since the plate became infected and had to be discarded.

## 3.8. Section 3:7

### 3.8.1. Possible seasonal changes in faecal output of infective larvae (Expt 13)

Many parasites exhibit a biological rhythm which can be related to their biology, the ecology of the host or vector, or climactic conditions (reviewed by Hawking, 1975). Graham (1939) suggested there was a seasonal variation in the production of free-living adults from faecal cultures of *S.ratti*, which could be correlated to changes in the environmental conditions to which the host was exposed. Therefore data on the output of third-stage larvae over 2 years from faecal cultures of both strains of *S.ratti* and *S.venezuelensis* was collected to discover if there was any obvious pattern of this sort.

#### 3.8.1.1. Materials and methods

Faecal cultures of each strain of *S.ratti* and *S.venezuelensis* were routinely prepared on day 7 post-infection (Section 2:1, Chapter 2, page 30). The number of infective larvae produced per gm of faeces, the total number of larvae which could theoretically have been produced by the total amount of faeces collected, and the percentage of larvae non-motile was estimated over the course of two years.

#### 3.8.1.2. Results

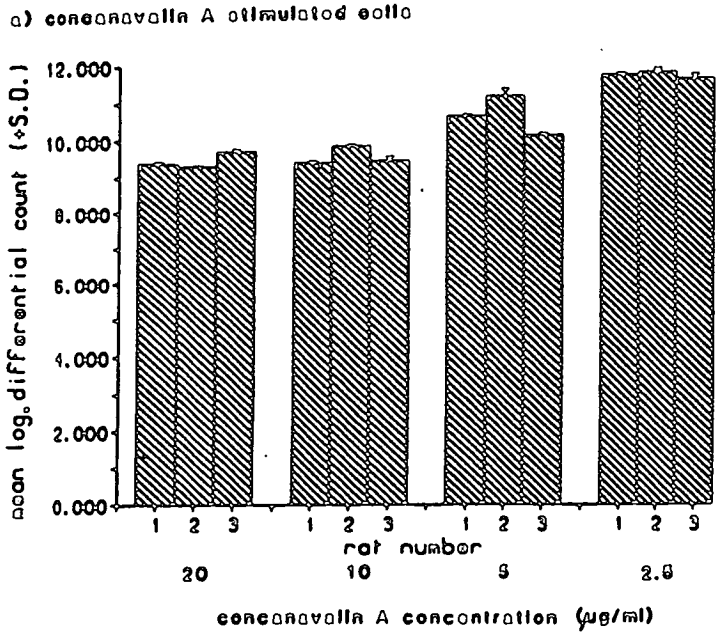
The number of larvae produced by the total amount of faeces collected per rat over the two years for the three parasites is shown in Figs.2:1, 2:2 and 2:3

Fig.3:4

Mesenteric lymph node cells obtained from different rats were exposed to various concentrations of mitogen (Fig.3:4a) or serum-free medium (unstimulated controls, Fig.3:4b) in order to find the concentration which would produce a high level of blastogenesis.

Fig.3:4

Effect of varying concanavalin A concentration, Expt 10



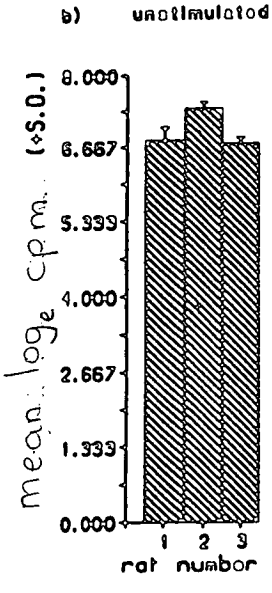



Fig.3:5

The effect of varying the time at which the mesenteric lymph node cells were harvested was investigated to find out what effect it had on the level of mitogenic (Fig.3:5a)and unstimulated (Fig.3:5b)stimulation obtained.

Fig.3:5

Effect of harvesting on different days, Expt 11

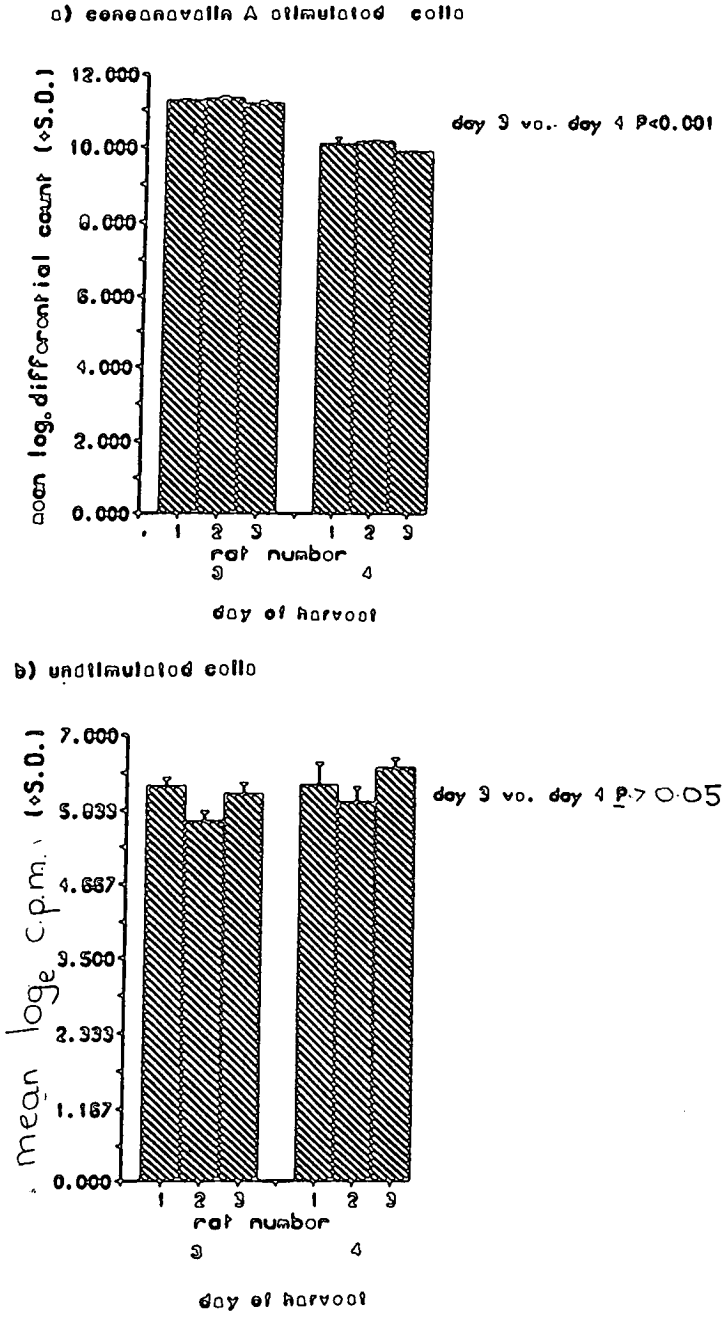


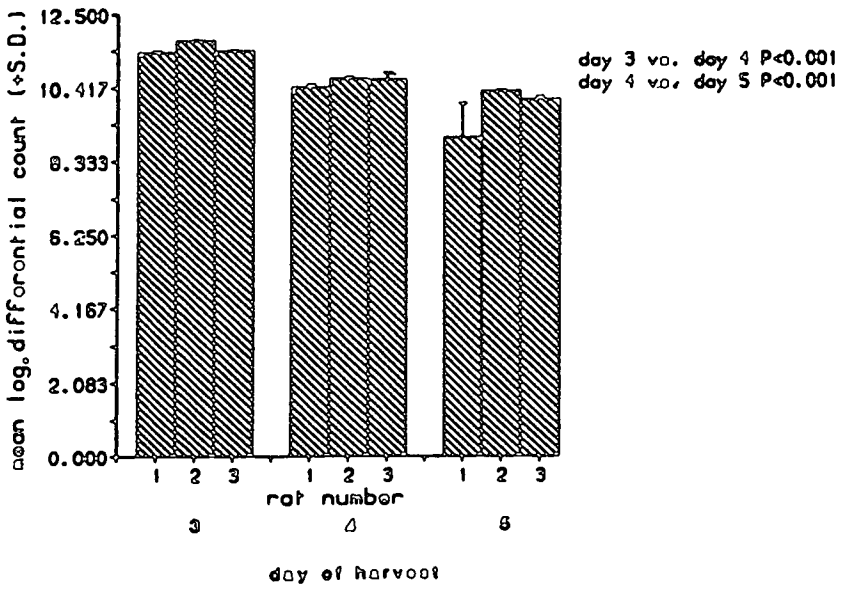
Fig.3:6

Cells exposed to either concanavalin A (Fig.3:6a) or serum-free medium (unstimulated controls, Fig.3:6b) were harvested on days 3, 4 and 5 of culture, to discover what effect the length of time spent on culture had on the level of stimulation obtained.

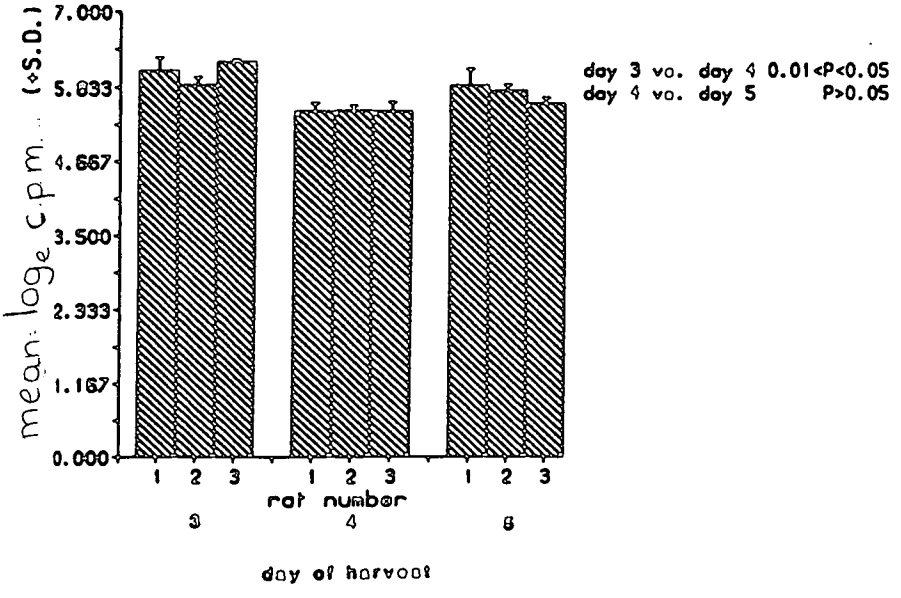
Fig. 3:6

Effect of harvesting on different days, Expt 12

a) concanavalin A stimulated cells




b) unstimulated cells



(Appendix 2, page 303). Although there are some gaps in the data there did not seem to be any consistent pattern in the seasonal variation in the output of infective larvae, since the peak recoveries occurred at different times each year for each parasite. (Other data from this study are shown in Tables 2:6(i), 2:6(ii) and 2:6(iii), Appendix 2, page 294).

3.9. Section 3:8

Properties of some of the data

The properties of the type of data collected routinely in this study have been explored by Wilson and Simpson (1981). The statistical methods adopted in this context are those recommended by these authors. Two types of data were new, however, and it was necessary to establish the correct procedure for their analysis. The number of eggs in the uteri of worms proved to be an important criterion in assessing resistance. On the face of it, such data, being discontinuous and having a low mean, could well have a frequency distribution significantly divergent from normal. It was nessary to test this possibility before they were analysed according to the principles of parametric statistics.

In addition, the data obtained from the lymphocyte blastogenesis assays presented another problem in that the variances of the original observations from different treatments were quite obviously proportional to their means. This property violates a condition necessary for the application of analysis of variance. The standard procedure to overcome this difficulty is to convert the individual variates to their logarithms. Samples of the relevant data were transformed in this way and inspected to see whether the offending relationship between mean and variance had disappeared. The frequency distribution of the transformed numbers was also tested to confirm that it

matched the normal curve.

1. Method:

Differential counts per minute obtained from different treatments within the lymphocyte blastogenesis assays were converted to their natural logarithms. These were scrutinised for any obvious relationship between mean and variance. Samples of the transformed results with similar means, as well as samples of control and experimental observations of eggs *in utero*, were tested using the "Minitab" computer package (Edinburgh Regional Computing Centre). This generates a new set of data which are normally distributed and have the same mean and standard deviation as the test data. The two sets of data are compared for correlation and a P value is obtained from tables using the program's calculated correlation coefficient and the number of replicates per treatment. This shows how well the test data fitted a normal distribution.

2. Results

The variances of samples of log transformed counts from blastogensis assays were not proportional to their means. Neither type of data under test had frequency distributions significantly different from normal (Tables 2:7(i) and 2:7(ii), Appendix 2, page 306). On these grounds it is feasible to apply parametric techniques of analysis to both.

The general methods used in this Chapter can be found in the relevant section of Chapter 2 given below:-

i) method for infecting rats by subcutaneous injection or skin-application Section 2:10, page 47.

ii) method for counting the number of worms and the number of eggs *in utero* per worm Section 2:10, page 50.

iii) method for estimating the number of third-stage larvae Section 2:1, page 30.

iv) statistical analysis of results, Section 2:11, page 51.

All results are shown in Appendix 2, pages 290-306).

## 3.10. Summary

i) Housing rats either singly, or in pairs, did not affect the proportion of the dose of *S.ratti* (homogonic strain) recovered on day 20 post-infection, when differences in the immune response would have been detected.

ii) Rats carrying *S.ratti* (homogonic strain) seemed to be incapable of re-infecting themselves from their own contaminated faeces.

iii) Results following surgical transfer of adult *Strongyloides spp.* suggested that a donor: recipient ratio of 1:4 should be possible; that worms should be transferred within two hours of the donor's death to ensure maximum survival; and that the viability of female worms taken from different donors was homogeneous.

iv) "Auto-infection" of rats by *Strongyloides spp.* was unlikely in this experimental framework.

v) Cell transfer medium did not affect the proportion of *S.ratti* (homogonic strain) recovered from rats post-infection and a 1:3 donor to recipient ratio should be feasible in cell transfer experiments if rats are to receive $2.5 \times 10^8$ cells each.

vi) Conditions for lymphocyte transformation assays would include 15% human serum in the cell medium, concanavalin A at a concentration of 2.5μg/ml for stimulation, and cells would be harvested on day 4 of culture.

vii) No apparent seasonal variation in the production of infective *Strongyloides spp.* from faecal cultures was detected.

viii) The data from counting eggs in the uterus of parasitic worms and the

transformed lymphocyte blastogenesis data could be legitimately analysed by parametric statistical techniques.

# CHAPTER 4

# COURSE OF INFECTION OF AN EXACT DOSE OF LESS THAN 100 THIRD-STAGE LARVAE OF *STRONGYLOIDES SPP.*

## 4.1. Introduction

In order to ensure that any cross-immunity demonstrated in reciprocal challenge experiments using the various parasites was specific in origin, it would be necessary to challenge rats when all the responses against the primary infection had subsided. In that case, at challenge, it would be the presence of immunological memory against the priming agent that would be investigated, and any cross-protection found after homologous and heterologous challenge would be because the two parasites shared common functional antigens. Therefore initially it was nessary to know:-

i) the course of a primary infection for each strain of *S.ratti* and *S.venezuelensis*.

ii) whether there was any strain or species-specific type of primary infection. In the experiments rats were infected with an exact dose of less than 100 third-stage larvae. Previous workers (Sheldon, 1937; Moqbel and Denham, 1977;1978; and Moqbel and McLaren, 1980) had found that the majority of *S.ratti* adults were expelled between days 25-30 post-infection, however the doses used in their experiments were a factor of ten greater than those planned. Therefore it was possible that such a small infection may have elicited a less potent anti-worm response and thus be able to survive much longer in the host.

## 4.2. Materials and methods

Three experiments were carried out using the protocol shown in Fig.4:1, page 83.

### 4.2.1. Expts. 1 and 2, Course of infection of an exact dose of <100 homogonic *S.ratti* third-stage larvae

There were three amendments to the general protocol in experiment 1:-

i) Day 8 injection controls were omitted and the experimental day 8 rats were also used as the "cosmic controls" (page 51).

ii) Results for day 32 post-infection come from 8 rats. A day 38 sample time was intended but 3 rats did not recover from the effects of the anaesthetic, and one rat was found to be pregnant; therefore the remaining two rats were included in the day 32 treatment.

iii) Only worm numbers were counted. Experiment 2 was a repeat of experiment 1, carried out to confirm results and also to ascertain the worm distribution within the intestine, and the number of eggs *in utero* per worm during the course of infection. The general protocol shown in Fig.4:1 was followed with no alterations. The mean exact dose at priming was 97.5 third-stage larvae in experiment 1, 92.5 for skin-application rats and 89.0 for injection control animals in experiment 2.

### 4.2.2. Expt. 3, Course of infection of an exact dose of <100 third-stage heterogonic *S.ratti* larvae

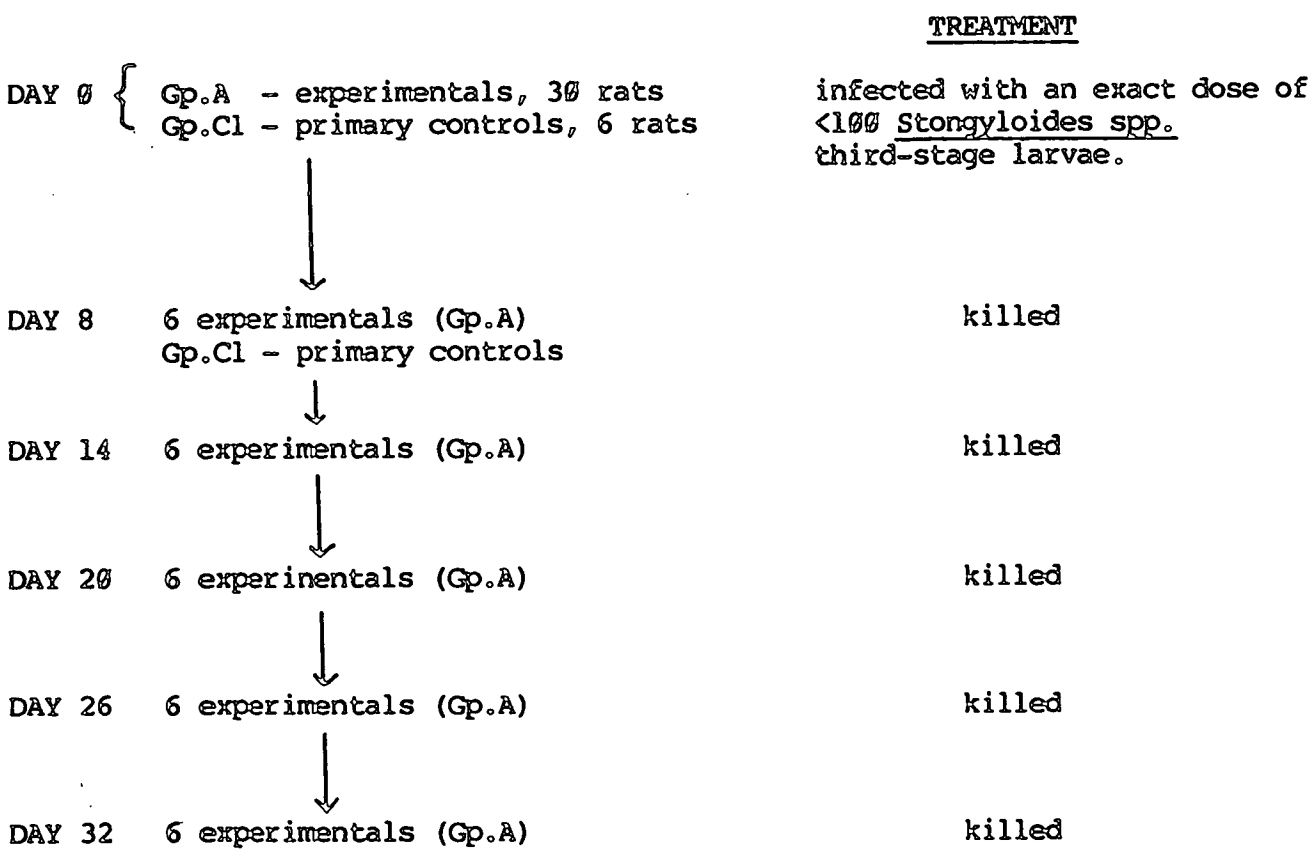
The protocol used was identical to that shown in Fig 4:1. The mean exact

Fig.4:1

Thirty-six rats (Gps.A and C1) were infected with an exact dose of <100 infective *S.ratti* larvae; Gp.A by skin-application and Gp.C1 by subcutaneous injection. Eight days later six rats from Gp.A and the primary controls, Gp.C1 (used to assess the viability of the larvae) were killed. The remaining animals in Gp.A were killed in batches of six on days 14, 20, 26 and 32 so that the course of a primary infection of the parasite could be followed. The number of parasitic female worms present, their distribution along the intestine and the number of eggs *in utero* per worm was recorded for each rat.

Fig.4:1

General protocol used in experiments to study course of a primary infection



dose was 95.1 for skin-application rats and 95.2 for injection control animals.

4.2.3. Expt. 4, Proportion of the dose recovered on day 8 post-infection, in rats infected with *S.venezuelensis*

Since *S.venezuelensis* was a new parasite to this laboratory there was no background information on its worm burden post-infection. Therefore this initial experiment was carried out to find what proportion of an exact dose of less than 100 third-stage larvae would be recovered on day 8 post-infection in Wistar rats of the Edinburgh colony.

Eight rats were infected with an exact dose of <100 third-stage *S.venezuelensis* larvae, four by skin-application (mean exact dose 97.8 larvae) and four by subcutaneous injection without an anaesthetic (mean exact dose 94.8 larvae). Rats were killed on day 8 post-infection and their worm burden found.

The modes of infection, methods used to count worm numbers, their distribution within the intestine and the mean number of eggs *in utero* per worm are given in Section 2:10, Chapter 2, page 47. Statistical analyses were as described in Section 2:11, page 51.

4.3. Results [Expts 1, 2, 3 and 4]

1. Proportion of the exact dose recovered

In both the homogonic and heterogonic strain of *S.ratti* there was a reduction in the number of adults recovered over the course of infection. By day 26 post-infection the majority of adults had been lost (results of Expts.

1-3, shown in Fig.4:2, pages 86-88). Results for days 8, 14 and 20 post-infection, only, were analysed since the mean proportion of the dose recovered on days 26 and 32 was much smaller. Surprisingly, in experiment 3 (Fig.4:2(iii), page 88), there was a significant difference in the proportion of the dose recovered from day 8 experimental rats and controls ($P<0.01$), the cause of which is unknown. Comparison of all three experiments showed there was a difference in the pattern of worm loss, since a two-way analysis of variance of "experiments" *versus* "days" revealed a significant variance attributable to interaction ($P<0.025$, Appendix 3:3a, page 309). If experiment three was omitted from the analysis, so that data for the homogonic strain only was analysed, this interaction disappeared, implying similar dynamics in those experiments. The significant differences in the proportion of the dose recovered between Expts 1 and 2 for each sample time ($P<0.001$, Appendix 3:3a, page 309) were consistent and not surprising, because the different infective larvae employed could have had a different potential for development (Wilson and Simpson, 1981). Only a very small proportion of the dose was recovered from rats infected with *S.venezuelensis*, regardless of the method of infection used (mean 0.056 ± 0.033 injection; 0.020 ± 0.005 skin-application). The reason for this was unclear, but it seemed pointless to study the course of infection of such a small number of adults.

2. The number of eggs *in utero* per worm

There was a reduction in the number of eggs *in utero* per worm for both *S.ratti* strains over the course of infection, until day 20 post-infection for the heterogonic strain, and day 26 post-infection for the homogonic strain, when it reached a minimum of approximately 2 eggs *in utero* per worm (Fig.4:2(ii)b and 4:2(iii)b, pages 87 and 88). Eggs *in utero* at the outset were approximately

Fig.4:2

Rats were infected with an exact dose of <100 third-stage *S.ratti* larvae and killed at various intervals post-infection to discover the length of the infection. Results for the homogonic strain (Expts 1 and 2) are shown in Fig.4:2(i) and 4:2(ii), whereas those for the heterogonic strain (Expt 3) are given in Fig.4:2(iii).

Fig.4:2

Egg and worm data over the course of infection of an exact dose of <100 third-stage S.ratti larvae, Expts 1, 2 and 3

1) Expt 1 homogonic S.ratti

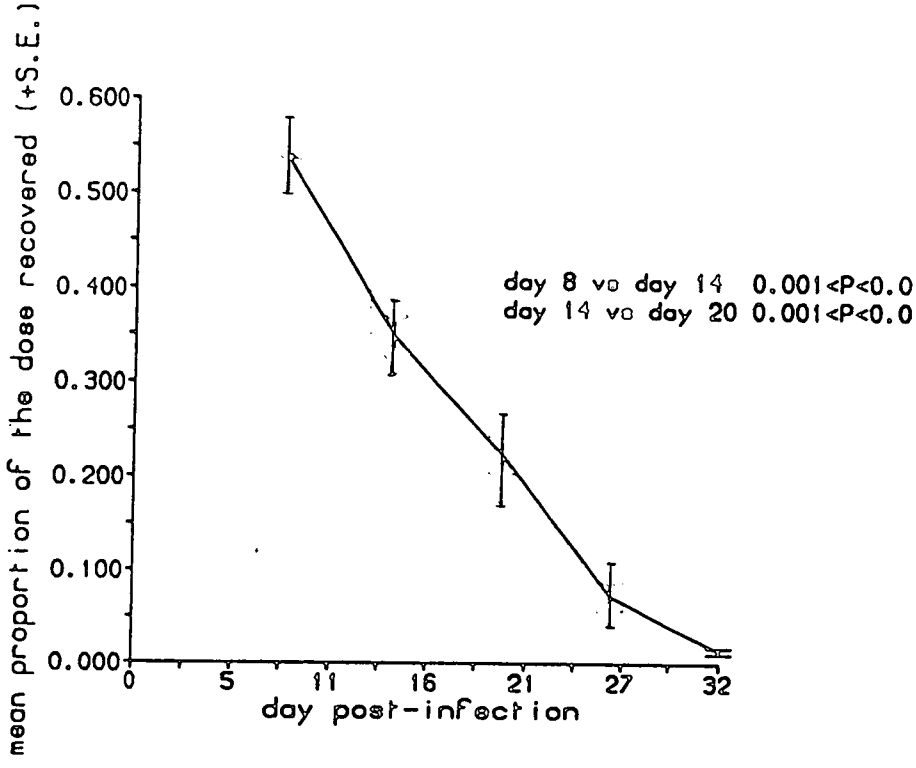
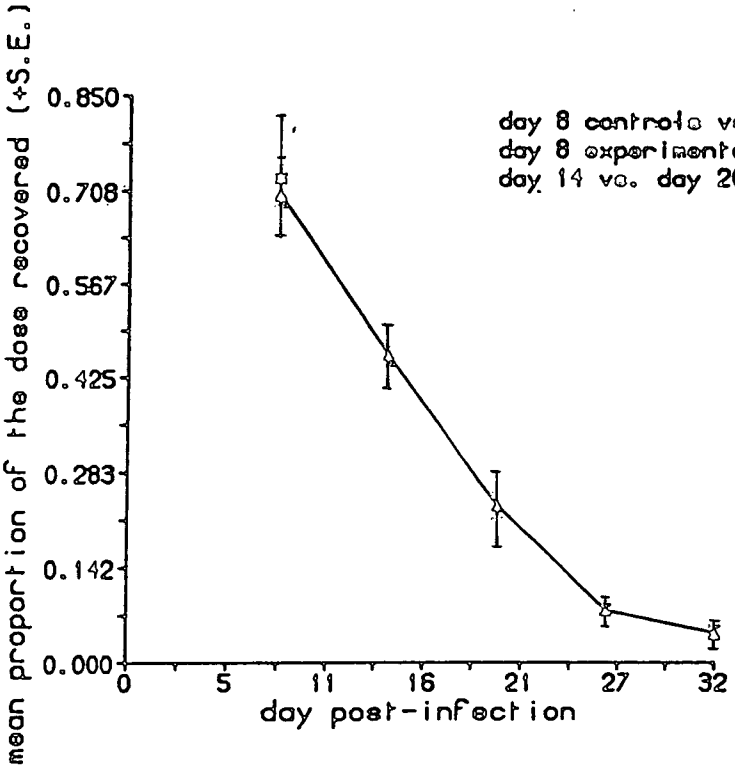


Fig.4:2 (continued)

II) Expt 2 homogonic S.ratti

a) worm data




b) egg data

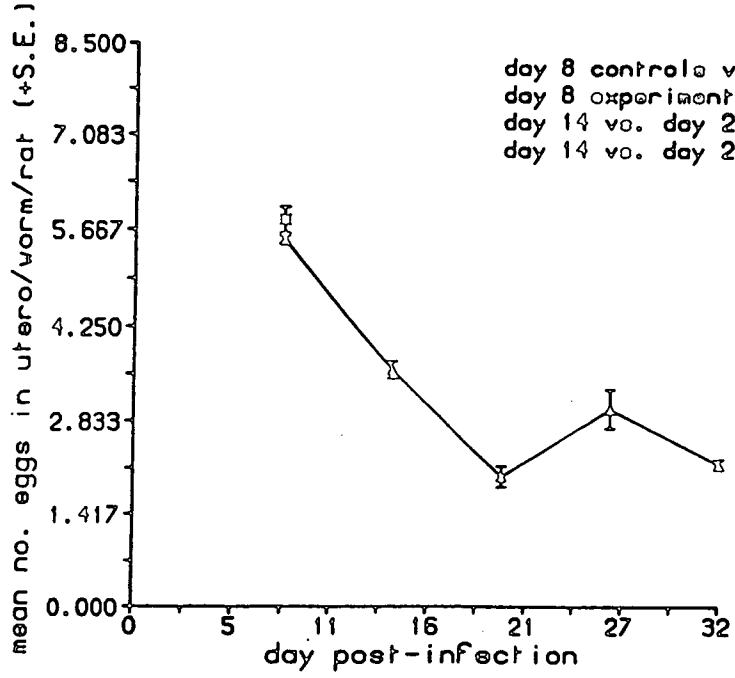
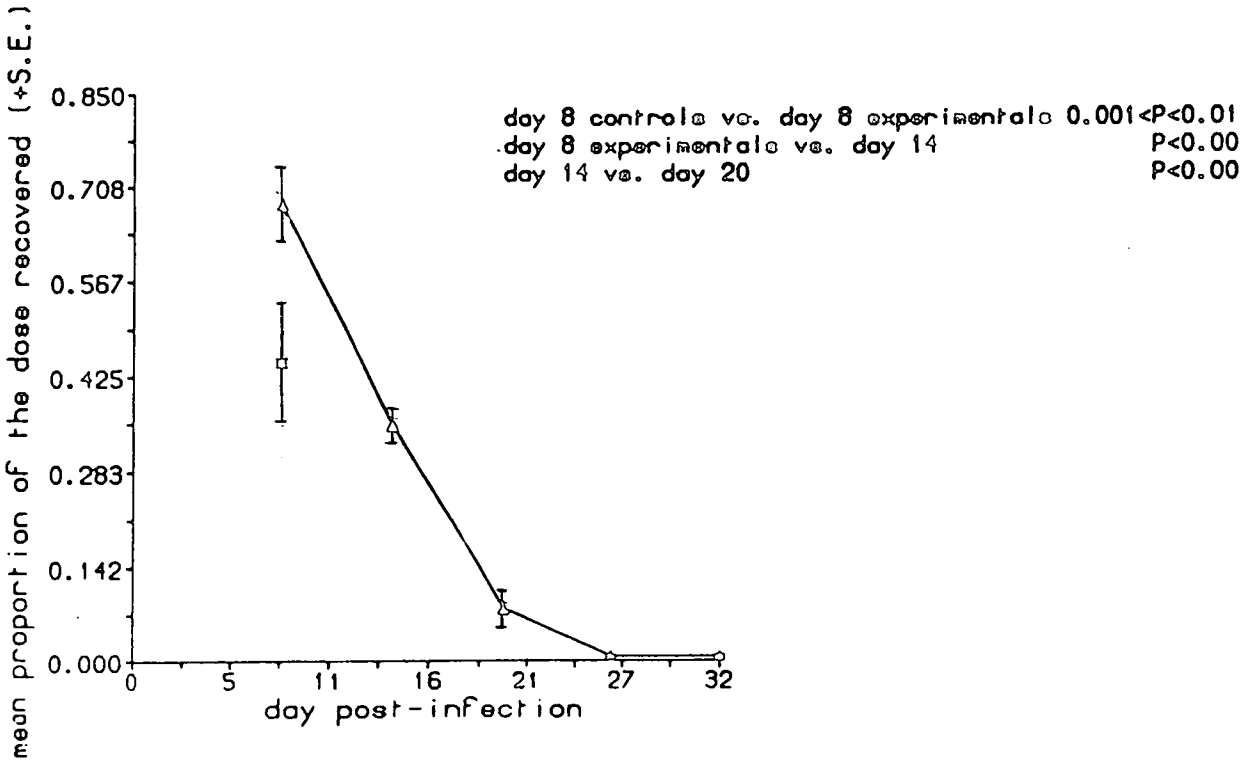


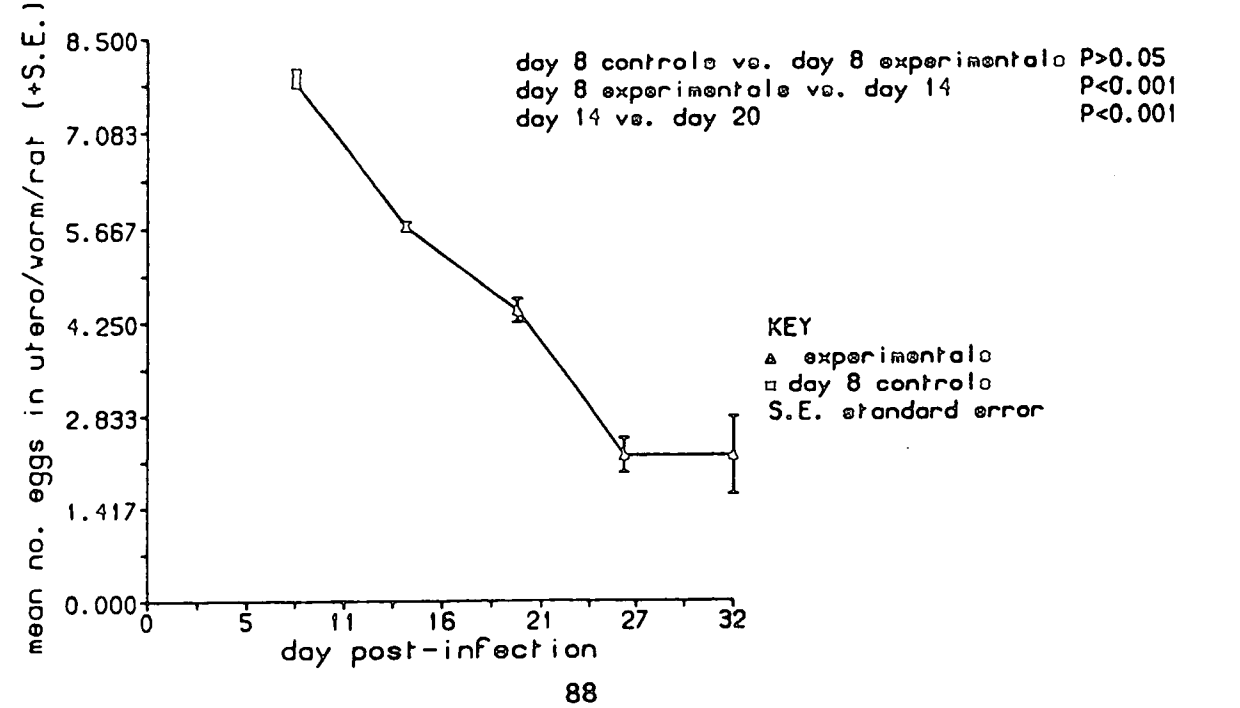
Fig.4:2 (continued)

III) Expt 3 heterogonic S.ratti

a) worm data




b) egg data



8 and 6 for the homogonic and heterogonic strains respectively.

3. Distribution of adults along the intestine

It was difficult to determine whether a posterior migration of adults had occurred late post-primary infection since results are based on the location of a maximum of 8 worms (Tables 3:2(i), (ii) and (iii), Appendix 3, page 308.). There was a possible movement towards the rear of adult homogonic *S.ratti* worms on day 32 post-infection, since a greater proportion of the parasites was recovered from section 8, but further studies would be necessary to confirm this. There was a more plausible posterior migration of adult heterogonic *S.ratti*, since a greater proportion of the worms was recovered from section 8 on day 20 post-infection, and on days 26 and 32 post-infection the majority of parasites were recovered from section 4 of the intestine instead of the more usual site of section 1. Further studies using a greater number of replicates per treatment for the later sample times are necessary to show whether this posterior movement of worms is a reality.

There did seem to be a difference in the distribution of adult *S.venezuelensis* and *S.ratti*, since on day 8 post-infection adult *S.ratti* were recovered as far as section 7 of the intestine (Tables 3:2(i), (ii) and (iii), Appendix 3, page 308), whereas adult *S.venezuelensis* were only recovered from sections 1 and 2. Further studies are necessary to confirm this observation.

4. Rate of reduction in the proportion of the dose recovered and in the number of eggs *in utero* per worm over the course of primary infection

Exponential coefficients were calculated from the data in order to follow

the rate of worm loss and the reduction in the number of eggs *in utero* per worm over the course of *S.ratti* infection using the equation :-

$$P_t = P_o e^{-xt}$$

where:-

| | | |
|---|---|---|
| $P_t$ | = | proportion of the dose recovered at time "t" |
| $P_o$ | = | proportion of the dose recovered on day 8 post-infection |
| t | = | time in days |
| x | = | exponential coefficient |

The coefficients varied greatly at each sample time when the rate of reduction in the proportion of the dose was considered, whereas the rate of reduction in the number of eggs *in utero* per worm seemed to be more constant (Fig.4:3, page 91 ). Thus the force responsible for the reduction in the proportion of dose recovered was either a single unit which varied in intensity over the course of infection, or a number of units, which acted together to cause the observed reduction. Whereas if the reduction in the number of eggs *in utero* per worm is considered the factor responsible for its changes seemed to be constant over the course of infection (see General Discussion, Chapter 10). However further experimentation would be necessary to confirm these findings.

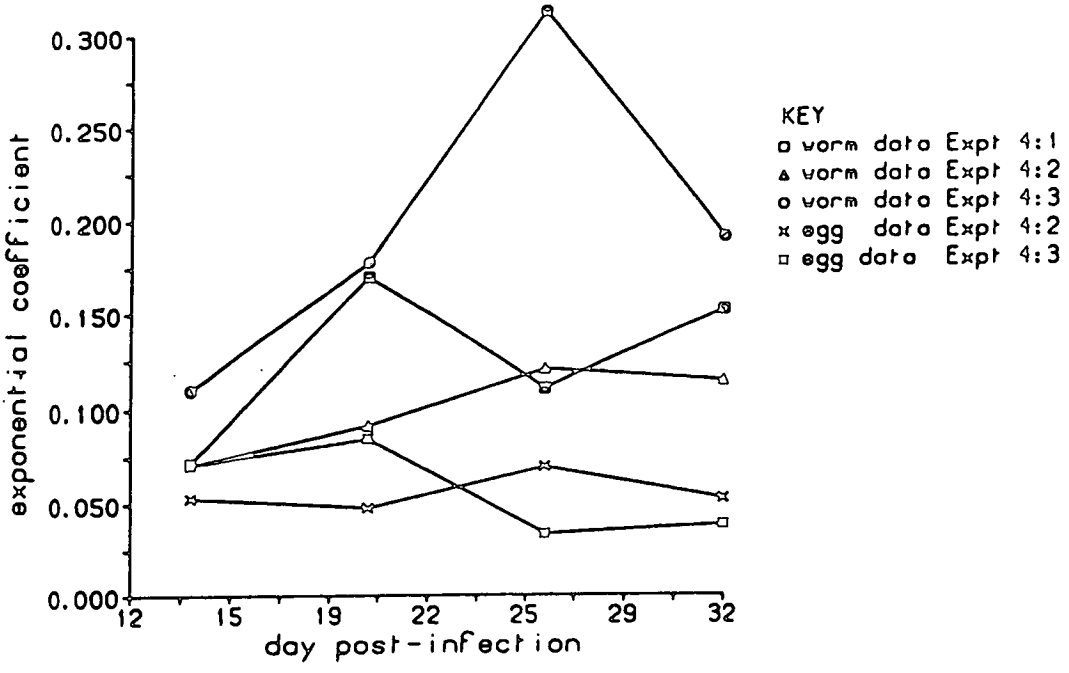
The results obtained in this section are shown in Appendix 3, which includes statistical analyses of the data (page 307 ).

Fig.4:3

The figure shows the rate of reduction in the worm and egg data over the course of infection with *S.ratti* (homogonic and heterogonic strains), using results fron Expts 4:1, 4:2 and 4:3 (see text).

Fig.4:3

Calculated exponential coefficients showing rate of reduction in egg and worm data over the course of infection with S.ratti, Expts 1, 2 and 3



## 4.4. Summary

The results of experiments described in this section showed that the majority of adult worms from a primary infection of *S.ratti* (homogonic and heterogonic strains), initiated with an exact dose of <100 third-stage larvae, were expelled by day 26 post-infection in Edinburgh colony Wistar rats. There seemed to be a difference in the pattern of worm loss for the two strains of *S.ratti*. Calculation of exponential coefficients suggested that the rate of reduction in the number of eggs *in utero* per worm seemed to be fairly constant over the course of infection, whereas the rate of reduction in the proportion of the dose recovered varied over the course of infection. Infection of Edinburgh colony Wistar rats with an exact dose of <100 third-stage *S.venezuelensis* larvae led to the recovery of an insignificant worm burden on day 8 post-infection, regardless of the mode of infection used; and there seemed to be a difference in the distribution of adult *S.ratti* and *S.venezuelensis* worms.

# CHAPTER 5

## THE AMOUNT OF RECIPROCAL CROSS-IMMUNITY BETWEEN THE TWO STRAINS OF *S.RATTI* AND *S.VENEZUELENSIS*

### 5.1. Introduction

The experiments carried out in this section had two main aims:-

a) to discover whether small exact doses of less than 100 third-stage larvae of the three parasites would stimulate an effective resistance to homologous challenge infection with a similar small dose.

b) to investigate the amount of reciprocal inter-specific and intra-specific cross-immunity between the two strains of *S.ratti* and *S.venezuelensis*.

For reasons which will appear later in this chapter and also in "The course of primary infection" (Chapter 4, page 81) some of the possible cross-reactions could not be studied comparatively.

In order to make clear decisions about the immunological relationship of the three parasites, rats were not re-infected until four weeks post-priming. Previous results had shown that, by then, the majority of worms from a priming dose were expelled from the intestine (results of Expts 1-3, Chapter 4, page 84). Thus any non-specific inflammatory response against the primary infection should have subsided by this time.

Previous workers have shown that a strong resistance to *S.ratti* challenge infection can be induced in rats (Sheldon, 1937; Moqbel and Denham, 1977; Murrell, 1980), and mice (Dawkins and Grove, 1980, 1982). None of these

studies has involved the use of more than one strain and all have been based on the use of doses 5-10 times larger than those used in this study, which were less likely to pick up subtle differences in the host's immune response for a number of reasons (see Discussion).

Very little work has been carried out on the level of cross-resistance between *Strongyloides* species. Dawkins and Grove (1982) found that priming with *S.stercoralis* (infective larvae) protected mice against heterologous challenge with *S.ratti*, measured as a suppression in the number of larvae produced per gm faeces in comparison to control mice. This is a very artificial system since neither *S.ratti* nor *S.stercoralis* occur naturally in the mouse. Moreover, reciprocal challenge experiments were impossible because *S.stercoralis* is unable to complete its life cycle in this host. A major advantage of the experimental system used in this investigation was that all three parasites were in their natural host.

## 5.2. Materials and methods

The general protocol shown in Fig.5:1 (page 96) was followed, with various amendments for individual experiments. 36 rats were divided into 5 main groups:-

Gps.A and B - 20 rats, infected by skin-application with an exact dose of <100 third-stage *Strongyloides spp.* larvae. Four weeks later ten animals (Gp.A) were challenged by the same method with a similar small dose of the homologous parasite (X), and the remainder (Gp.B) with the heterologous parasite (Y).

Gp.C1 - 6 rats, primary controls - infected with an exact dose of <100

third-stage larvae of the same parasite (X) used at priming.

Gp.C2 - 5 rats, secondary controls - infected at challenge with an exact dose of <100 third-stage larvae of the homologous parasite (X) used at priming.

Gp.C3 - 5 rats, secondary controls - infected at challenge with an exact dose of <100 infective larvae of the heterologous parasite (Y).

Controls were infected either by skin-application with anaesthesia or by subcutaneous injection without anaesthesia. All rats were killed either on day 8 post-primary infection or day 8 post-challenge. The number of adult worms present, their distribution within the intestine, and the mean number of eggs *in utero* per worm for each rat was found. Detailed descriptions of the methods used are given in Section 2:10 and 2:11, Chapter 2, pages 47 and 51 respectively).

### 5.2.1. Expts 1, 2 and 3, Homologous challenge with homogonic strain of *S.ratti*

The same procedure described in the general protocol (page 94) was followed with a few ammendments (Fig.5:2 for Expt 1; page 97, Fig.5:3 for Expts 2 and 3, page 98) since rats were only challenged with the homologous parasite. Expts. 2 and 3 were run in conjunction to confirm the results of Expt 1. To economise on the number of animals used, rats were primed together with larvae from the same pool, and shared priming controls. However at challenge each experiment had its own controls and animals were challenged with larvae from different pools. Controls from all three experiments were infected by subcutaneous injection.

Fig.5:1

Rats in Gps.A, B and C1 were infected with an exact dose of <100 infective *S.ratti* larvae of the homogonic strain. The larvae were administered by skin-application to animals in Gps.A and B whereas they were either injected subcutaneously or applied by skin-application to those in Gp.C1. To check the viability of the larvae used the priming controls (Gp.C1) were killed on day 8 post-infection. The challenge was administered on day 28 of the primary infection when Gp.A were infected with the homologous parasite (X) and Gp.B with the heterologous strain of *S.ratti* or species (parasite Y). Corresponding controls were infected at the same time (Gps.C2 and C3). Rats in all treatments were infected with an exact dose of <100 infective larvae given by skin-application to the experimentals, and either by skin-application or subcutaneous injection to the challenge controls. Animals were killed 8 days later and the proportion of the dose recovered, the distribution of worms along the intestine, and the number of eggs *in utero* per worm found.

Fig.5:1

General protocol

| | | | TREATMENT |
|---|---|---|---|
| DAY 0 | Gps.C2 and C3 | | untreated |
| | Gps.A and B | - experimentals | primed with |
| | Gp.C1 | - priming controls | parasite X |
| DAY 8 | Gp.C1 | - priming controls | killed |
| DAY 28 | | | challenge |
| | Gp.A | - homologous challenge | infected with parasite X |
| | Gp.B | - heterologous challenge | infected with parasite Y |
| | Gp.C2 | - secondary controls | infected with parasite X |
| | Gp.C3 | - secondary controls | infected with parasite Y |
| DAY 36 | ---------- ALL RATS ---------- | | killed |

Rat numbers used

| Group | Expt | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 |
| Gp.A = | 10 | 10 | 9 | 10 | 9 | 10 |
| Gp.B = | 10 | 9 | 10 | 10 | 10 | 10 |
| Gp.C1 = | 5 | 6 | 5 | 4 | 5 | 5 |
| Gp.C2 = | 5 | 5 | 5 | 4 | 5 | 5 |
| Gp.C3 = | 6 | 4 | 5 | 5 | 5 | 5 |

Fig.5:2

On day 0 rats in Gps.A and C1 were infected with an exact dose of <100 infective homogonic strain *S.ratti* larvae, given by skin-application to rats in Gp.A and injected subcutaneously to rats in Gp.C1. Eight days later the priming controls (Gp.C1) were killed to assess the viability of the larvae used. On day 28 post-infection rats in Gps.A, C2 (skin-application controls) and C3 (injection controls) were infected with an exact dose of <100 third-stage homogonic strain *S.ratti* larvae. Animals were killed 8 days later and the proportion of the dose recovered from the homologous challenge treatment (Gp.B) and its corresponding controls (Gps.C1 and C2) compared to find if any resistance to *S.ratti* infection was induced with such a small priming dose.

Fig 5:2

Protocol for Expt 5:1

| | | TREATMENT |
|---|---|---|
| DAY 0 | 18 rats untreated (Gps.C2 and C3)<br>Gp.A - homologous challenge, 12 rats<br>Gp.C1 - priming controls, 6 rats | priming with an exact dose of <100 third-stage homogor strain S.ratti larv |
| DAY 8 | Gp.C1 - priming controls | killed |
| DAY 28 | Gp.A - homologous challenge<br>Gp.C2 - challenge controls (skin-application), 12 rats<br>Gp.C3 - challenge controls (injection), 6 rats | challenge with an exact dose of <100 infective homogonic S.ratti larvae |
| DAY 36 | ALL RATS | killed |

Fig.5:3

Animals in Gps.A1, A2 and C1 were infected with an exact dose of <100 third-stage homogonic *S.ratti* larvae by skin-application (Gps.A1 and A2) or subcutaneous injection (Gp.C1). The priming controls (Gp.C1) were killed 8 days later to give an estimate of the viability of the larvae used. On day 28 post-infection animals were challenged with an exact dose of <100 infective larvae of the homologous parasite with a different larval pool being used to challenge animals in Expts 2 and 3. The experimental animals in each experiment were infected by skin-application of the larvae whereas controls were injected subcutaneously with them. Eight days later all the rats were killed and the amount of resistance expressed in each experiment was found by comparing the proportion of the dose recovered and the number of eggs *in utero* per worm of the challenged treatments (Gps.A1 and A2) with their corresponding controls (Gps.C2 and C3).

Fig.5:3

Protocol for experiments 5:2/3

| | | | TREATMENT |
|---|---|---|---|
| DAY 0 | 10 rats untreated (Gps.C2 and C3) | | priming with homogonic strain of S.ratti |
| | Gps.A1 and A2 | - homologous challenge, 24 rats | |
| | Gp.C1 | - priming controls, 6 rats | |
| DAY 8 | Gp.C1 | - priming controls | killed |
| DAY 28 | | | challenge with homogonic strain of S.ratti |
| | Gp.A1 | - homologous challenge, Expt 2, 12 rats | |
| | Gp.A2 | - homologous challenge, Expt 3, 12 rats | |
| | Gp.C2 | - challenge controls, Expt 2, 5 rats | |
| | Gp.C3 | - challenge controls, Expt 3, 5 rats | |
| DAY 36 | ---------- ALL RATS ---------- | | killed |

### 5.2.2. Expts 4 and 5, Homologous vs heterologous challenge: homogonic strain of *S.ratti* priming animals

There was one changes to the general protocol described on page 95, and shown in (Fig.5:1, page 96). In Expt 4 the number of third-stage larvae produced per gm of faeces by the challenge treatments and their corresponding controls was found on days 6-8 post-challenge. Three rats were randomly chosen from each group and placed in individual grid cages on day 5 post-challenge. Faecal collections were made every day and individual cultures for each rat were set up on days 6,7 and 8 post-challenge. The number of third-stage larvae produced per gm of faeces and the theoretical number of larvae which could have been produced from the total amount of faeces collected was noted. The method of culture is given in Chapter 2 (Section 2:1, page 30).

Controls in Expt 4 were infected by subcutaneous injection of infective larvae whereas those in Expt 5 were infected by skin-application of larvae.

### 5.2.3. Expts 6 and 7, Homologous vs heterologous challenge: heterogonic strain of *S.ratti* priming animals

The reciprocal challenge infection to that described in Expts 4 and 5 was carried out with no adjustments to the general protocol (given on page 95, and shown in Fig.5:1, page 96). Controls in Expt 6 were infected by subcutaneous injection of third-stage larvae, whereas those in Expt 7 were infected by skin-application.

5.2.4. Expts 8 and 9, Homologous vs heterologous challenge: *S.venezuelensis* priming animals

There were no adjustments to the general protocol described on page 95 and shown in Fig.5:1, page 96. In experiment 8 the homogonic strain of *S.ratti* was used as the heterologous parasite whereas in Expt 9 the heterogonic strain was utilised. Controls in both experiments were infected by skin-application of infective larvae of the relevant parasite.

The doses used in each experiment are shown Appendix 4, pages 312-318.

5.3. Results

1. Worm data

In all experiments where *S.ratti* was used to immunise and challenge rats, there was a significant reduction in the proportion of the dose recovered ($\underline{P}<0.001$), and in the number of eggs *in utero* per worm ($\underline{P}<0.001$) after homologous and heterologous challenge compared with the corresponding controls, regardless of the strain used to prime or challenge animals (Fig.5:4, Table 5:1a and Table 5:1b, pages 101 and 102).

However, if *S.venezuelensis* was used to prime rats either no immunity was elicited or there seemed to be a degree of immunosuppression since a significantly greater proportion of the dose was recovered from heterologous challenged rats in Expt 8 and 9 ($\underline{P}<0.001$), and from homologous challenged rats in Expt 9 ($0.01<\underline{P}<0.05$, Table 5:1c, page 103) compared with corresponding controls. Adult *S.venezuelensis* were recovered on day 36 post-primary infection from the heterologous challenged groups in Expts 8 and

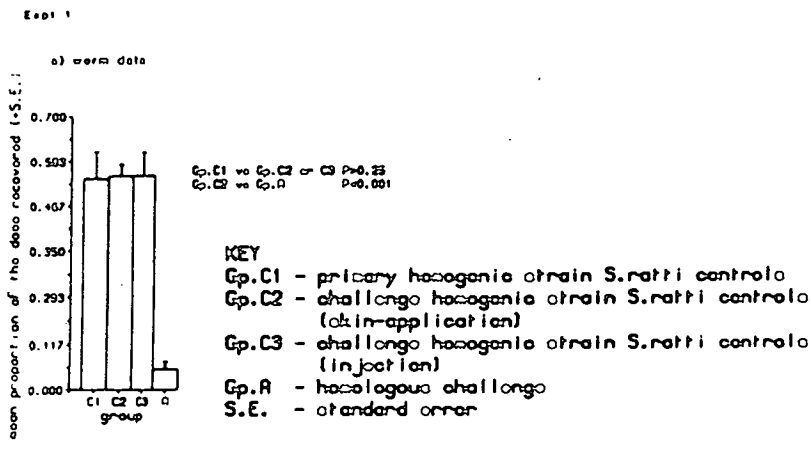
Fig.5:4

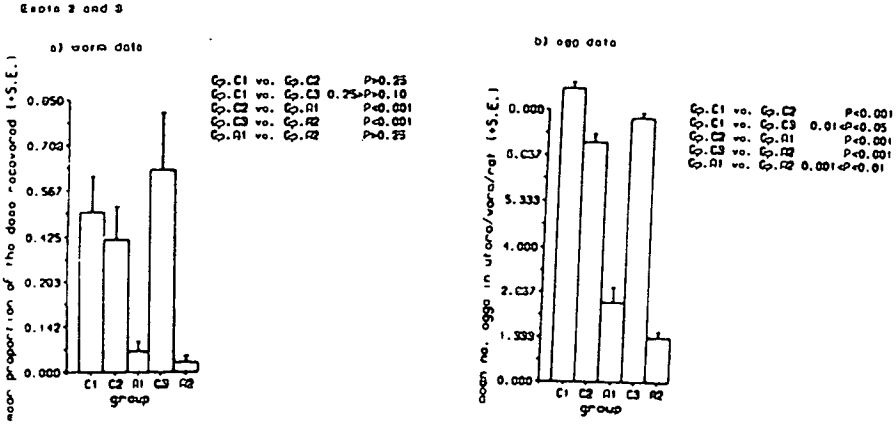
Rats were primed with an exact dose of <100 third-stage homogonic strain *S.ratti* larvae and then challenged on day 28 post-primary infection with a similar small dose. The amount of resistance produced was found by comparing the proportion of the dose recovered (Expts 1, 2 and 3) and the number of eggs *in utero* per worm (Expts 2 and 3) from immunised rats (Gp.A in Expt 1; Gp.A1 in Expt 2; and Gp.A2 in Expt 3) and their corresponding challenge controls (Gps.C2 and C3; Gp.C2; Gp.C3: respectively).

Fig.5:4

Effect of homologous challenge with the homogonic strain of S.ratti,

Expts 1, 2 and 3




KEY
Gp.C1 - primary homogenic S.ratti controls
Gp.C2 - challenge homogenic S.ratti controls, Expt 2
Gp.C3 - challenge homogenic S.ratti controls, Expt 3
Gp.A1 - homologous challenge, Expt 2
Gp.A2 - homologous challenge, Expt 3
S.E. - standard error

Table 5:1

Rats were infected with an exact dose of <100 infective larvae of either the homogonic strain of *S.ratti*, the heterogonic strain of *S.ratti* or *S.venezuelensis*. Four weeks later the animals were challenged with a similar small dose of the homologous (Gp.A) or heterologous (Gp.B) parasite. The amount of resistance elicited in the primed animals was found by comparing the proportion of the dose recovered and the number of eggs *in utero* of the experimentals (Gps.A and B) with their corresponding controls (Gp.C2 and C3, respectively). Table 5:1(a) homogonic strain of *S.ratti* priming rats, challenge with the homologous and heterologous strain. Table 5:1(b) heterogonic strain of *S.ratti* priming animals, challenge with the homologous and heterologous strain. Table 5:1(c) *S.venezuelensis* immunising animals, challenge with the homologous parasite and either homogonic strain of *S.ratti* (Expt 8) or heterogonic strain of *S.ratti* (Expt 9).

Table 5:1

a) Homologous vs heterologous challenge: homogenic strain of S.ratti priming rats

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 4 | homologous challenge | Gp.A | 0.124 | 10 | 0.110 | 1.402 | 10 | 1.111 |
| | heterologous challenge | Gp.B | 0.066 | 10 | 0.086 | 1.616 | 7 | 0.846 |
| | priming controls | Gp.C1 | 0.565 | 5 | 0.318 | 8.311 | 5 | 0.905 |
| | challenge controls homogonic S.ratti | Gp.C2 | 0.407 | 5 | 0.022 | 7.566 | 5 | 0.586 |
| | challenge controls heterogonic S.ratti | Gp.C3 | 0.534 | 6 | 0.208 | 5.875 | 6 | 0.331 |
| | mean exact dose | priming | homogonic S.ratti | | skin-application | | | 91.10 |
| | | | | | injection | | | 87.00 |
| | | challenge | homogonic S.ratti | | skin-application | | | 97.10 |
| | | | | | injection | | | 89.40 |
| | | | heterogonic S.ratti | | skin-application | | | 95.50 |
| | | | | | injection | | | 81.67 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | 0.25>P>0.10 | 0.01<P<0.05 |
| Gp.C2 vs C3 | 0.25>P>0.10 | P<0.001 |
| Gp.C2 vs A | P<0.001 | P<0.001 |
| Gp.C3 vs B | P<0.001 | P<0.001 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | homologous challenge | Gp.A | 0.079 | 10 | 0.069 | 0.901 | 8 | 0.556 |
| | heterologous challenge | Gp.B | 0.030 | 9 | 0.036 | 0.801 | 6 | 1.102 |
| | priming controls | Gp.C1 | 0.541 | 6 | 0.069 | 7.300 | 6 | 0.441 |
| | challenge controls homogonic S.ratti | Gp.C2 | 0.568 | 5 | 0.156 | 6.319 | 5 | 0.565 |
| | challenge controls heterogonic S.ratti | Gp.C3 | 0.438 | 4 | 0.055 | 4.803 | 4 | 0.434 |
| | mean exact dose | priming | homogonic S.ratti | | skin-application | | | 95.62 |
| | | challenge | homogonic S.ratti | | skin-application | | | 95.65 |
| | | | heterogonic S.ratti | | skin-application | | | 94.08 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.05 | 0.01<P<0.05 |
| Gp.C2 vs C3 | 0.25>P>0.10 | P<0.001 |
| Gp.C2 vs A | P<0.001 | P<0.001 |
| Gp.C3 vs B | P<0.001 | P<0.001 |

Table 5:1

b) Homologous vs heterologous challenge: heterogonic strain of S.ratti priming rats

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 6 | homologous challenge | Gp.A | 0.034 | 9 | 0.043 | 1.200 | 5 | 0.633 |
| | heterologous challenge | Gp.B | 0.320 | 10 | 0.061 | 2.259 | 10 | 0.650 |
| | priming controls | Gp.C1 | 0.492 | 5 | 0.358 | 6.030 | 5 | 1.199 |
| | challenge controls heterogonic S.ratti | Gp.C2 | 0.540 | 5 | 0.116 | 6.180 | 5 | 0.629 |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.663 | 5 | 0.133 | 7.400 | 5 | 0.875 |
| | mean exact dose | priming | heterogonic S.ratti | | skin-application | | | 95.55 |
| | | | | | injection | | | 84.67 |
| | | challenge | heterogonic S.ratti | | skin-application | | | 94.89 |
| | | | | | injection | | | 95.40 |
| | | | homogonic S.ratti | | skin-application | | | 94.80 |
| | | | | | injection | | | 98.40 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.25 | P>0.25 |
| Gp.C2 vs C3 | 0.25<P<0.10 | P<0.001 |
| Gp.C2 vs A | P<0.001 | P<0.001 |
| Gp.C3 vs B | P<0.001 | P<0.001 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | homologous challenge | Gp.A | 0.010 | 10 | 0.013 | 0.800 | 5 | 0.767 |
| | heterologous challenge | Gp.B | 0.159 | 10 | 0.085 | 2.233 | 10 | 0.788 |
| | priming controls | Gp.C1 | 0.471 | 4 | 0.159 | 6.363 | 4 | 0.350 |
| | challenge controls heterogonic S.ratti | Gp.C2 | 0.368 | 4 | 0.063 | 5.778 | 4 | 0.586 |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.524 | 5 | 0.063 | 6.602 | 5 | 0.274 |
| | mean exact dose | priming | heterogonic S.ratti | | skin-application | | | 97.52 |
| | | challenge | heterogonic S.ratti | | skin-application | | | 96.67 |
| | | | homogonic S.ratti | | skin-application | | | 96.40 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | 0.10>P>0.05 | 0.10>P>0.05 |
| Gp.C2 vs C3 | 0.25>P>0.10 | P<0.001 |
| Gp.C2 vs A | P<0.001 | P<0.001 |
| Gp.C3 vs B | P<0.001 | P<0.001 |

Table 5:1

c) Homologous vs heterologous challenge: S.venezuelensis priming rats

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 8 | homologous challenge | Gp.A | 0.060 | 9 | 0.042 | | | |
| | heterologous challenge | Gp.B | 0.458 | 10 | 0.140 | 6.807 | 10 | 1.109 |
| | residual S.venezuelensis | Gp.D | 0.057 | 8 | 0.058 | | | |
| | priming controls | Gp.C1 | 0.047 | 5 | 0.062 | | | |
| | challenge controls S.venezuelensis | Gp.C2 | 0.023 | 5 | 0.023 | | | |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.247 | 5 | 0.156 | 6.638 | 5 | 0.603 |

| | | | | |
|---|---|---|---|---|
| mean exact dose | priming | S.venezuelensis | skin-application | 96.65 |
| | | | injection | 98.40 |
| | challenge | S.venezuelensis | skin-application | 95.22 |
| | | | injection | 93.20 |
| | | homogonic S.ratti | skin-application | 97.10 |
| | | | injection | 88.80 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.25 | |
| Gp.C1 vs D | P>0.25 | |
| Gp.C2 vs A | 0.25>P>0.10 | |
| Gp.C3 vs B | P<0.001 | P>0.25 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9 | homologous challenge | Gp.A | 0.098 | 10 | 0.051 | | | |
| | heterologous challenge | Gp.B | 0.690 | 10 | 0.122 | 3.455 | 10 | 0.596 |
| | residual S.venezuelensis | Gp.D | 0.048 | 9 | 0.032 | | | |
| | priming controls | Gp.C1 | 0.023 | 5 | 0.018 | | | |
| | challenge contols S.venezuelensis | Gp.C2 | 0.041 | 5 | 0.021 | | | |
| | challenge controls heterogonic S.ratti | Gp.C3 | 0.359 | 5 | 0.100 | 5.125 | 5 | 0.354 |

| | | | | |
|---|---|---|---|---|
| mean exact dose | priming | S.venezuelensis | skin-application | 96.15 |
| | | | injection | 97.20 |
| | challenge | S.venezuelensis | skin-application | 97.20 |
| | | | injection | 92.60 |
| | | heterogonic S.ratti | skin-application | 92.70 |
| | | | injection | 93.00 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | 0.25>P>0.10 | |
| Gp.C1 vs D | 0.25>P>0.10 | |
| Gp.C2 vs A | 0.01<P<0.05 | |
| Gp.C3 vs B | P<0.001 | P< 0.001 |

N.B.

S.venezuelensis adults from the primary infection were recovered from the heterologous challenged rats (Gp.B). The proportion of the primary infection dose recovered from these animals is shown separately as Gp.D in this table.

9 (shown as the residual *S.venezuelensis* treatment, Gp.D, Table 5:1c, page 103). They were distinguished from adult *S.ratti* worms using a morphological criterion (Section 2:1, Chapter 2, page 23). Therefore adults recovered from homologous challenged rats in Expts 8 and 9 could be derived from the primary or challenge infection. Thus the significant increase in the proportion of the dose recovered from the homologous challenge group compared to controls in Expt 9 could be the result of challenge worms simply adding to the adults already present from the primary infection. However, this does not explain why more adult *S.ratti* worms were recovered from heterologous challenged rats compared to their controls in Expts 8 and 9. It should be noted that the proportion of the dose recovered from the secondary controls was lower than that obtained in previous experiments (Expts 1, 2, 3, 4 and 5 for the homogonic strain; and Expts 6 and 7, for the heterogonic strain; shown in Fig.5:4 and Table 5:1a and b, pages 101 and 102) but there is no logical reason to believe that this had a fundamental impact on the outcome. If immunosuppression had occurred in these experiments no anti-worm response would have been expected but in Expt 9 there was a significant reduction in the number of eggs *in utero* per worm for the heterologous challenged treatment compared to its control ($P<0.001$, Table 5:1c, page 103). A corresponding reduction was not found in Expt 8 but this could be related to the use of different strains in the two experiments.

**2. Quantitative comparison of homologous and heterologous challenge**

The degree of protection resulting from homologous and heterologous challenge was compared by calculating resistance quotients for the two treatments within each experiment (Fig.5:5, pages 105 and 106). These gave a clear indication of the effectiveness of each treatment since the height of the bar is directly proportional to the level of resistance produced. If homogonic

Fig.5:5

The amount of resistance expressed against homologous and heterologous challenge infection was found by calculating the resistance quotients from the egg and worm data collected. The height of the bar for both treatments is directly proportional to the amount of protection produced. In Expts 4 and 5 the homogonic strain of *S.ratti* was used at priming and rats were challenged with either the homologous (Gp.A) or heterologous strain (Gp.B). The reciprocal experiment was carried out in Expts 6 and 7 and the heterogonic strain was used to immunise animals. *S.venezuelensis* was the priming agent in Expts 8 and 9, animals were challenged with the homologous parasite (Gp.A) and either the homogonic (Gp.B, Expt 8) or heterogonic strain of *S.ratti* (Gp.B, Expt 9).

Fig.5:5

Quantitative comparison of the effect of homologous and heterologous challenge on the egg and worm data, Expts 4-9

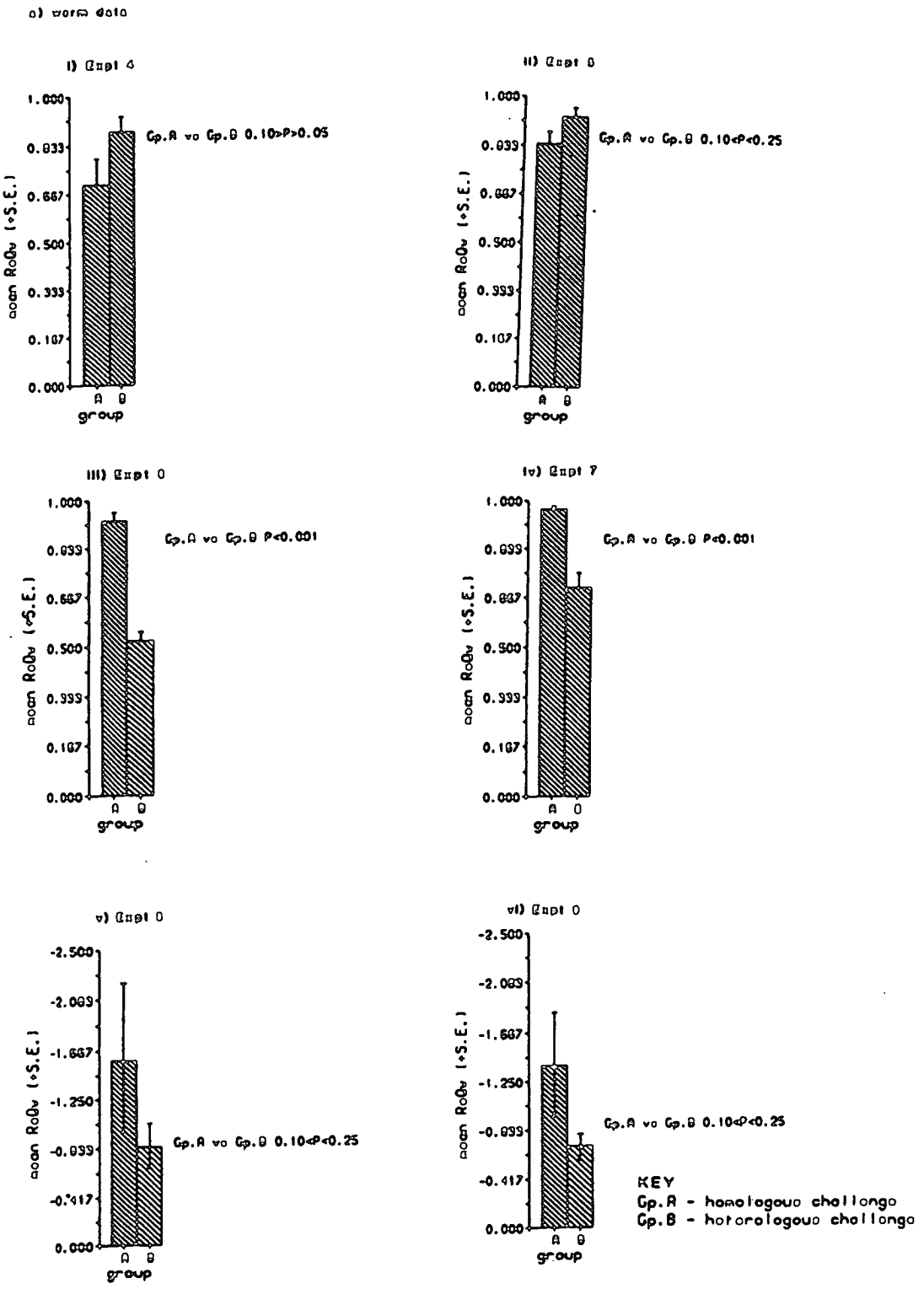
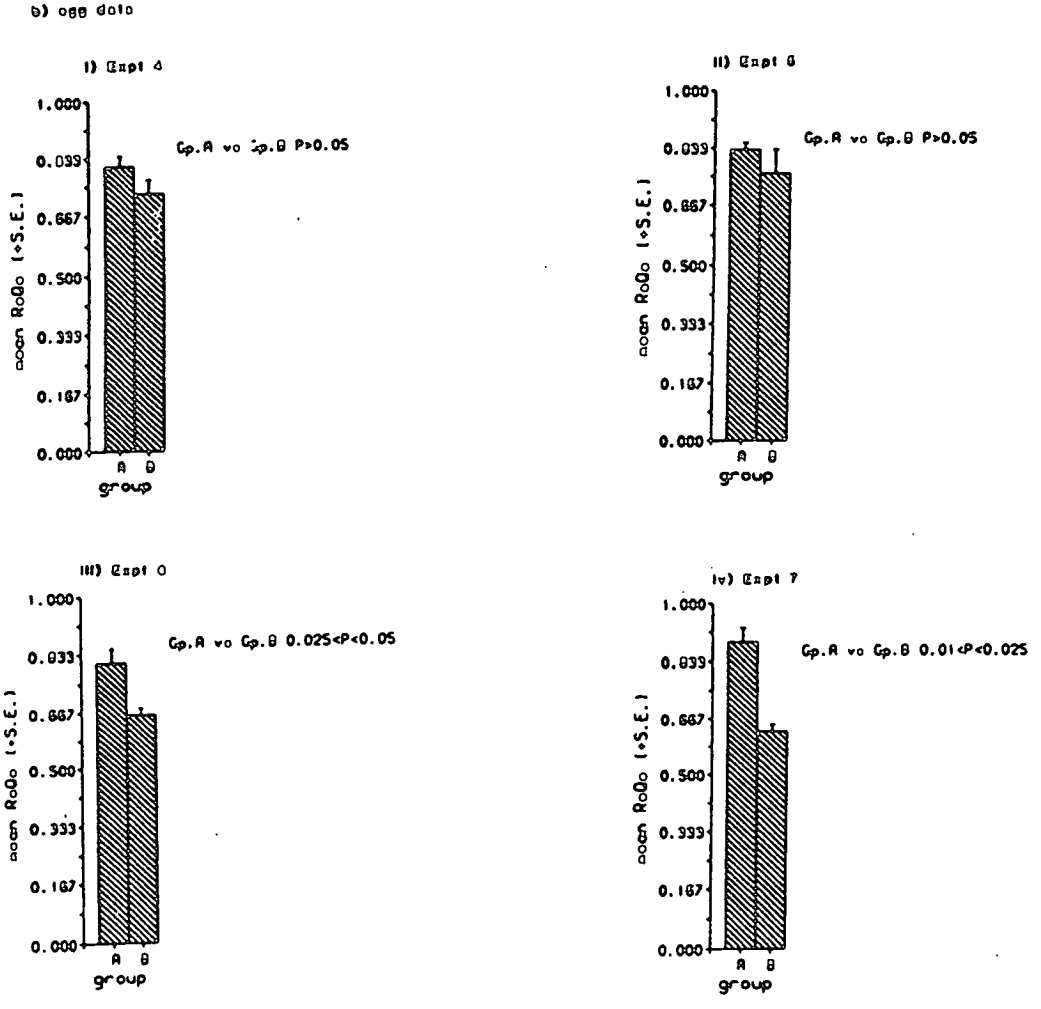


Fig.5:5 (continued)



*S.ratti* was used to prime rats there was no significant difference in the level of immunity elicited by homologous or heterologous challenge (Fig.5:5(i) and (ii), Expts 4 and 5, pages 105 and 106). However, in the reciprocal challenge experiment, homologous challenge with heterogonic *S.ratti* produced a significantly greater level of immunity expressed as a greater reduction in the proportion of the dose recovered ($\underline{P}<0.001$), and a greater reduction in the number of eggs *in utero* per worm [$0.025<\underline{P}<0.05$, Expt 6; $0.01<\underline{P}<0.025$, Expt 7, see Fig.5:5 (iii) and (iv), page 105 and 106). These results contrast with those obtained when *S.venezuelensis* was used to prime rats since there was no reduction in the worm data in either experiment (shown by the negative Resistance Quotient, "ReQw", in Fig.5:5(v) and (vi), Expts 8 and 9, page 105).

3. Worm fecundity post-challenge

In one of the experiments information was collected in an attempt to answer the question "does a reduction in the number of eggs *in utero* per worm reflect a reduction in worm fecundity ?" It was feasible that female worms succumbing to the host's immune response may have laid eggs at a faster rate to offset any reduction in the number they produced. Thus fewer eggs *in utero* could actually be consistent with an <u>increase</u> in fecundity as much as the reverse. Therefore data was collected to discover if there was any relationship between the number of third-stage larvae produced per gram of faeces per worm (used as a measure of worm fecundity) with the number of eggs *in utero* per worm (used as a measure of the host's antiworm response throughout the study) post-challenge.

There did seem to be both a reduction in the number of third-stage larvae produced per gram of faeces per worm and in worm fecundity (Table 5:2, page

109) for the challenged treatments compared with their corresponding controls. But the high variability within the results, possibly because of the low number of replicates per treatment, did not allow firm conclusions to be made. The estimated relative reduction in the number of eggs *in utero* per worm was not proportional to the number of third-stage larvae produced per gram of faeces per worm (compare "$Ratio_1$" and "$Ratio_2$", Table 5:2, page 109) since, after homologous challenge there was a greater reduction in the number of eggs compared to the reduction in the number of third-stage larvae produced; and the reverse was found after heterologous challenge. Thus the observed change in the number of eggs *in utero* per worm is possibly not entirely accounted for in terms of a reduction in worm fecundity.

4. Worm distribution post-challenge

There may have been a posterior migration of adult *S.ratti* worms after homologous and heterologous challenge (regardless of strain used at priming) but since results are based on the relative position of 15 worms or less (minimum 1), it was impossible to make any firm conclusions (Tables 4:2(i)-(vi), Appendix 4, page 313 ). In experiments 8 and 9 where results were based on the relative position of forty, or more adults, there was a posterior migration of adult *S.ratti* worms in the heterologous challenge treatments compared with control data (Tables 4:2(vii) and 4:2(viii), Appendix 4, page 316 ). However, other criteria had shown that no measureable resistance was induced in these experiments (see above). These results suggest there was a difference in the longitudinal distribution of the two *Strongyloides spp.* within the intestine. Adult *S.ratti* worms were recovered from up to section 6 of the gut whereas *S.venezuelensis* worms were only recovered from sections 1 and 2, even in the heterologous challenge treatments. However this judgement was only

Table 5:2

Ratios 1 and 2 were calculated to discover whether there was any relationship between the the reduction in the number of eggs *in utero* per worm obtained in challenged treatments and worm fecundity.

Table 5:2

Comparison of worm fecundity, measured as number of third-stage S.ratti larvae produced per gm. of faeces per adult, and number of eggs in utero per worm on day 8 post-challenge

| Treatment | Group | mean no.L3 /gm faeces/ worm | n | SD | mean no. eggs in utero/worm/ rat | ratio1 $\frac{a}{b}$ | ratio2 $\frac{c}{d}$ |
|---|---|---|---|---|---|---|---|
| challenge controls homogonic S.ratti | Gp.C2 | 7.61 (b) | 3 | 4.31 | 6.319 (d) | | |
| homologous challenge (with homogonic S.ratti) | Gp.A | 5.31 (a) | 3 | 4.35 | 0.901 (c) | 0.698 | 0.143 |
| challenge controls heterogonic S.ratti | Gp.C3 | 2.67 (b) | 3 | 1.39 | 4.803 (d) | | |
| heterologous challenge (with heterogonic S.ratti) | Gp.B | 0.00 (a) | 3 | 0.00 | 0.801 (c) | 0.000 | 0.167 |

Where:-

ratio1 = $\frac{a}{b}$

ratio2 = $\frac{c}{d}$

Data in this table is taken from Expt 5

based on the location of a maximum of 10 *S.venezuelensis* worms. Results for each experiment in this Chapter are shown in Appendix 4, pages 312-318.

## 5.4. Summary

1. Priming with an exact dose of <100 third-stage *S.ratti* larvae elicited a strong protective response against challenge with a similar small dose, regardless of the strain used at either infection. This was demonstrated as a reduction in the proportion of the dose recovered, and a reduction in the number of eggs *in utero* per worm compared with corresponding controls.

2. Quantitative comparison of the level of resistance produced in rats after homologous and heterologous challenge showed that homologous challenge with heterogonic *S.ratti* produced a significantly greater level of immunity than heterologous challenge with homogonic *S.ratti*, assessed as a greater relative reduction in the proportion of the dose recovered, and in the number of eggs *in utero* per worm. However in the reciprocal challenge experiment there was no difference in the level of protection against either challenge treatment.

3. Priming rats with an exact dose of <100 third-stage *S.venezuelensis* larvae failed to immunise them against homologous challenge, or heterologous challenge with the homogonic strain of *S.ratti*, since there was no significant difference in the proportion of the dose recovered, or in the number of eggs *in utero* per worm, compared with corresponding controls. When heterogonic *S.ratti* was used at challenge a response could be detected in terms of a significant reduction in the number of eggs *in utero* per worm compared to the controls ($P<0.001$), even though there was no adverse effect on establishment of these same worms.

4. Data collected from one experiment suggested that the reduction in the number of eggs *in utero* per worm of *S.ratti* parasites obtained from challenged animals compared with corresponding controls may not be related

to a reduction in worm fecundity.

5. Measurements made on the distribution of adult worms along the intestine suggested that *S.ratti* worms were found in a more distal position in the intestines of challenged rats (irrespective of the strain used at priming) compared with corresponding controls; and that *S.venezuelensis* parasitic female worms had a more restricted distribution in the intestine compared with *S.ratti*. However, in both cases the observation was based on the location of only a few parasites so it may have no real significance.

CHAPTER 6

# STUDIES ON *STRONGYLOIDES VENEZUELENSIS*

## 6.1. Introduction

The aim of this series of experiments was to discover why only a few adult worms of this species developed from an exact dose in fully grown rats of the Edinburgh Wistar colony (see results of Expt 4, Chapter 4, page 84). The source of the problem could have been any of a number of factors including the age, sex , weight or strain of host, or the biology of the parasite, or simply bad luck at a first attempt in this laboratory.

The findings of Wertheim (1970a and b) suggested, however, that the strain of the host, mode of infection and the size of the dose could all be involved. Wertheim obtained 44% recovery of *S.venezuelensis* from young male rats of the "Subra" strain infected by intra-peritoneal injection with 250 third-stage larvae, but only 8% if rats were similarly infected with 1000 infective larvae. However in both cases there was a huge variation in her data (a range of 82-218 adults in rats infected with 250 third-stage larvae, and 14-180 following doses of 1000). In a study by Nolan and Katz (1981), a similar low parasite take was obtained in adult rats since only 3% of an inoculum of 4000 third-stage *S.venezuelensis* larvae was recovered from control rats.

In the light of Wertheim's report and from other considerations the following experiments were designed to investigate four parameters,namely; the effect of the age of the host, the size of dose, the mode of infection, and the use of different types of anaesthesia. The fate of third-stage larvae administered was also investigated, using radio-labelled infective larvae, in an

attempt to discover if *S.venezuelensis* larvae reached the host's intestine.

## 6.2. Materials and methods

### 6.2.1. Expt 1, Skin-application vs subcutaneous injection

Two groups of 4 adult rats were infected with an exact dose of less than 100 third-stage *S.venezuelensis* larvae, half the animals were infected by subcutaneous injection without anaesthesia, the remainder were infected by skin-application of the larvae whilst under Sagatal anaesthesia. Worm burdens were determined in the intestines of rats killed 8 days post-infection.

### 6.2.2. Expt 2, Ether vs Sagatal anaesthesia

Experiment 1 was repeated with one alteration, all rats were infected by skin-application of third-stage *S.venezuelensis* larvae, half the animals were under Sagatal anaesthesia, the others were maintained under ether anaesthesia for 10 minutes.

### 6.2.3. Expt 3, Size of dose

Six rats were infected by skin-application with 2000 third-stage *S.venezuelensis* larvae, i.e. a dose approximately 20 times larger than before. The number of adults present in the intestine on day 8 post-infection was recorded.

### 6.2.4. Expt 4, Age of host

Four three-week old and 4 adult rats were infected by skin-application of

an exact dose of <100 (mean 96.00) third-stage *S.venezuelensis* larvae. On day 8 post-infection the number of adults present in their intestines was recorded.

### 6.2.5. Expt 5, The effect of the age of the host on larval migration to the intestine

17 rats were divided into four groups:-

Gp. A - 5 adult rats (12 weeks old) - infected with *S.venezuelensis*

Gp. B - 5 adult rats (12 weeks old) - infected with *S.ratti*

Gp. C - 3 three-week old rats - infected with *S.venezuelensis*

Gp. D - 4 three-week old rats - infected with *S.ratti*

All rats were infected by skin-application with an estimated dose of 2500 ($^{75}$Se)-selenomethionine labelled infective larvae (see materials and methods, page 33) contained in approximately 0.3ml distilled water. The number of counts per minute per larva were 2.9 ± 0.4, for *S.venezuelensis*, and 2.0 ± 0.2, for *S.ratti*. Rats were killed 72 hours post-infection, at which time the proportion of the dose in their intestines was estimated.

### 6.2.6. Expt 6, Survival of worms and $^{75}$Se label over 8 days in a rat's intestine

Fifteen adult (12 weeks old) rats were infected by skin-application with an estimated dose of 2030 ($^{75}$Se)-selenomethionine labelled infective *S.venezuelensis* larvae (cpm per larva 3.7 ± 0.3). Seven of the rats were killed at 72 hours post-infection and the remainder were killed on day 8. The proportion of the labelled dose recovered at both times was calculated, but on

day 8 post-infection the number of adult worms present in the rats' intestines was also counted after measuring the level of radio-activity present. This would give an indication of the amount of attached label lost over the course of infection.

When rats were killed, the number of adults present, their distribution along the intestine, and the number of eggs *in utero* per worm per rat was found. The proportion of the dose in the anterior and posterior halves of the intestine was found in experiments using labelled larvae.

The methods of infection and the method of collecting worm and egg data is given in Chapter 2, Section 2:10, page 47. The method of obtaining ($^{75}$Se)-selenomethionine labelled infective larvae and estimating the proportion of the dose recovered is given in Chapter 2, Section 2:3, page 33. Edinburgh colony Wistar rats were used throughout experiments. The method of analysing the worm and egg data is given in Chapter 2, Section 2:11, page 51. The results from the experiments using labelled infective larvae were analysed in the same manner as the worm data using the proportion of the labelled dose recovered, in statistical tests.

## 6.3. Results

A low proportion of the exact dose of *S.venezuelensis* was recovered in Expts 1 and 2 suggesting that neither the mode of infection (mean subcutaneous injection 0.091 ± 0.0533; skin-application 0.092 ± 0.0597, Expt 1) nor the type of anaesthesia (mean Sagatal 0.034 ± 0.0135, ether 0.090 ± 0.0358, $P>0.10$, Expt 2) was responsible for the low adult burdens in the initial experiment (Expt 4, Chapter 4, page 84). Increasing the size of the infection dose did seem to have an affect, since 29% of the inoculum was recovered

from a dose of 2000 (compared with a maximum of 10% in Expts 1 and 2). No real conclusion can be drawn from these data since controls infected with an exact dose of *S.venezuelensis* larvae were omitted from the experimental protocol. Moreover, this result is in direct contradiction of those of Wertheim (1970a and b).

There seemed to be an age-related resistance to infection with *S.venezuelensis* since a significantly higher proportion of the dose was recovered as worms from three-week old rats compared with adults (12-week old rats) on day 8 (mean 0.298 ± 0.0401 three-week old rats, 0.013 ± 0.0080 adult rats, $P<0.001$, Expt 4). The results of Expt 5, where ($^{75}$Se)-selenomethionine labelled larvae were used to infect rats, mirrored this effect since a significantly greater level of radio-activity was recovered from the intestines of young rats compared with their adult counterparts on day 3 ($P<0.001$, Fig 6:1, page 118). An age-related resistance to infection was not demonstrated for *S.ratti* since a similar amount of radio-activity was present in the intestines of both young and adult rats ($P>0.25$, Fig 6:1, page 118 ). However, even though a larger proportion of the *S.venezuelensis* dose was recovered from three-week old rats in experiment 5, it was still lower than that obtained from similarly aged rats infected with *S.ratti* ($P<0.001$, Fig 6:1, page 118).

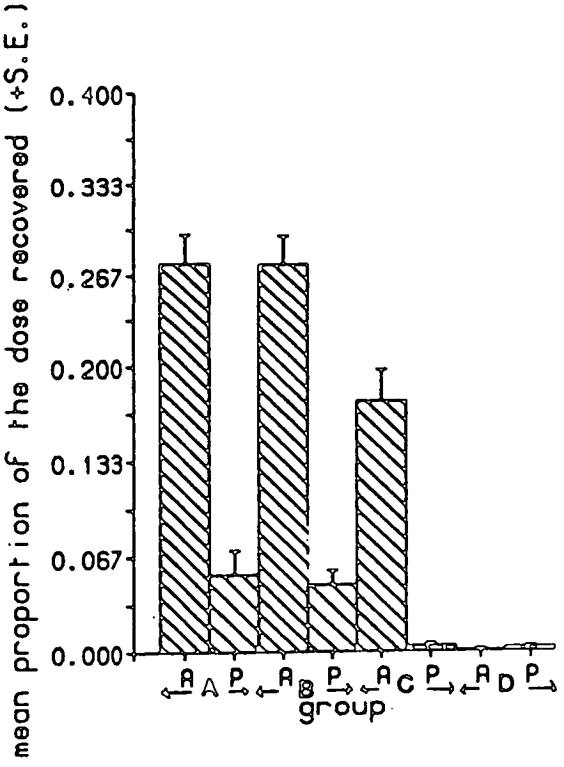
The results of experiment 6 (Fig 6:2, page 119), suggested that third-stage *S.venezuelensis* larvae were still reaching the intestine after 72 hours post-infection since a significantly higher proportion of the dose was recovered on day 8 post-infection compared with day 3 ($P<0.001$). This was contrary to expectation because attached label was likely to be lost during worm development. That label was lost overall in this experiment was clear,

Fig.6:1

Three-week old and adult rats were infected with an estimated dose of $^{75}Se$-labelled third-stage *S.venezuelensis* or *S.ratti* larvae (mean 2500). The amount of label present in their intestines on day 3 post-infection was recorded in order to ascertain whether *S.venezuelensis* larvae reached the intestine, and to find out if the age of the host had an effect.

Fig.6:1

Effect of age of the host, Expt 5




Gp.A vs. Gp.B P>0.25
Gp.C vs. Gp.D P<0.001
Gp.A vs. Gp.C P<0.001

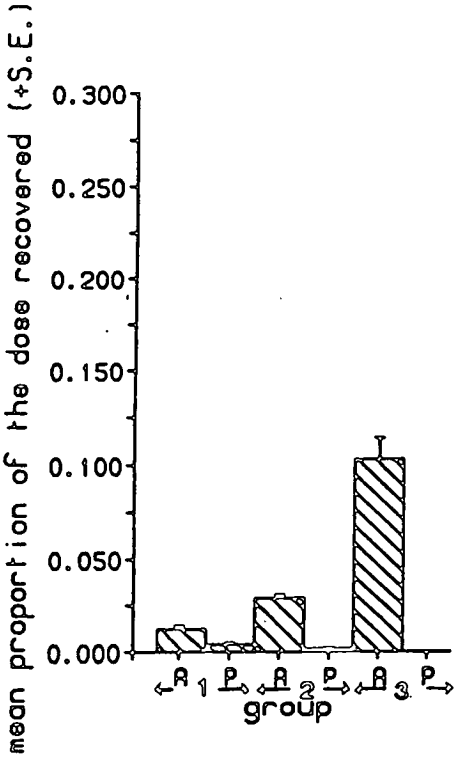
KEY
A - anterior intestine
P - posterior intestine
Gp.A - three-week old rats infected with homogenic strain S.ratti
Gp.B - adult rats infected with homogenic S.ratti
Gp.C - three-week old rats infected with S.venezuelensis
Gp.D - adult rats infected with S.venezuelensis
S.E. - standard error

Fig.6:2

Adult rats were infected with an estimated dose of radio-labelled *S.venezuelensis* larvae (mean 2030). The amount of label present in their intestines on day 3 and day 8 post-infection was recorded to discover if the age of the host had an effect and to find out if larvae were still reaching the intestine after 72 hours. On day 8 post-infection the actual numbers of worms present (Gp.3) was also counted to show how well the proportion of the larval dose recovered correlated with the proportion of the labelled dose recovered (Gp.2).

Fig.6:2

Proportion of the S.venezuelensis dose recovered at 72 hours and 8 days post-infection, Expt 6




Gp.1 vs. Gp.2 P<0.001
Gp.2 vs. Gp.3 P<0.001

KEY
Gp.1 - labelled dose recovered at 72hours
Gp.2 - labelled dose recovered at day 8
Gp.3 - larval dose recovered at day 8 (number of worms counted)
S.E. - standard error
A - anterior half of intestine
P - posterior half of intestine

since the worms at 8 days carried a maximum of approximately a third of what was originally attached to the third-stage larvae (Fig 6:2, page 119). Some of this loose label was recovered from the posterior half of the intestine on day 8 post-infection. Since no adults were actually found.

Throughout the 6 experiments adult *S.venezuelensis* were recovered from the anterior sections of the rats' intestines, mainly from section 1 in Expts 1-4 (Tables 5:2 (i)-(iv), Appendix 5, page 321 ) where the longitudinal distribution of adult worms along the intestine was noted.

Actual results for individual experiments are shown in Appendix 5, pages 319-321.

## 6.4. Summary

a) The proportion of the *S.venezuelensis* dose recovered in adu.t rats was unaffected by the mode of infection, or by the use of different anaesthetics. Expt 3 suggested that increasing the size of the infection dose may lead to an increased recovery. But this finding would have to be confirmed since controls, infected with a small exact dose of third-stage *S.venezuelensis* larvae, were omitted from the protocol.

b) There seemed to be an age-related resistance to infection with *S.venezuelensis* since a higher proportion of the dose was recovered from three-week old compared with adult (12 week-old) animals on days 3 and 8 post-infection.

c) The age related resistance probably affected the migratory stages of the life cycle as well as development in the intestine.

# CHAPTER 7

# DIFFERENT METHODS FOR STUDYING THE ANTIGENIC RELATIONSHIP OF THE TWO *STRONGYLOIDES SPP.*

## 7.1. Introduction

An unsuspected resistance was demonstrated in Wistar rats of the Edinburgh colony infected with *S.venezuelensis* in this study (results of Expts 4-6, Chapter 6, page 114), so that only a small proportion of an exact dose was recovered 8 days post-infection. In reciprocal cross-immunity experiments no protection against any sort of challenge was induced in rats primed with this low dose of *S.venezuelensis* (results of Expts 8 and 9, Chapter 5, page 100). It appeared, therefore, that the number of adults of this species present during a primary infection initiated in this way was below the threshold necessary to stimulate an immune response.

In order to circumvent the problem four methods of priming rats against *S.venezuelensis* were explored in the hope that one or more would provide an experimental framework in which cross-immunity using the two *Strongyloides spp.* could be studied.

## 7.2. Priming rats with heat-killed larvae [Expt 1]

Some workers, using dead nematode infective larvae to immunise animals against re-infection, have had limited success (reviewed by Clegg and Smith, 1978; and Lloyd, 1981). In contrast, resistance against *S.ratti* infection has been artificially produced in this manner by Sheldon (1937, 1939), and Murrell (1980). There seemed to be some specificity in the protection elicited, since

priming with *S.stercoralis* failed to immunise rats against infection with *S.ratti* (Sheldon, 1939). However their experimental design involved serial injections of increasing numbers of dead larvae, a total of 13 and 16 injections by Sheldon (1937, 1939, respectively), and 17 injections by Murrell (1980); a protocol which was too imprecise for the purposes of the present study and which necessitates finding a number of injection sites on the animal. Therefore the potency of single doses of 10,000 heat-killed third-stage larvae was tested followed by challenge 10 days later with an "exact" dose of live larvae. An initial study was carried out using homogonic *S.ratti* as the priming agent to see whether the method immunised animals, since previous experiments carried out in this study had shown that live infections of this parasite were capable of priming rats against re-infection (results of Expts 1, 2, 3, 4 and 5, Chapter 5, page 95).

### 7.2.1. Materials and methods [Expt 1]

35 rats were divided into 3 groups:-

Gp.A - 15 rats, immunised

Gp.B - 15 rats, day 20 challenge controls

Gp.C - 5 rats, day 8 secondary controls

Rats from group "A" were immunised by the subcutaneous injection of 10,000 heat-killed homogonic *S.ratti* larvae in 0.3ml distilled water, whilst under ether anaesthesia, using a 25G needle and a 1ml Plastipak syringe (see Section 2:4, Chapter 2, page 36, for the method of preparing heat-killed larvae). A second injection of 0.3ml distilled water was given with the same syringe to minimise the number of larvae left in the needle. On day 10

post-priming all rats were infected by skin-application with an exact dose of <100 third-stage homogonic *S.ratti* larvae. Animals in group "C" were killed on day 8 post-infection, and the remainder on day 20.

### 7.2.2. Results [Expt 1]

Priming with a single dose of 10,000 third-stage homogonic *S.ratti* larvae failed to produce any protection against homologous challenge infection ($P>0.25$, mean immunised rats 0.179 ± 0.018, day 20 challenge controls 0.211 ± 0.026). Therefore any further studies using this method were abandoned.

## 7.3. Priming with transferred adult worms [Expts 2-8]

Introduction of adult *S.venezuelensis* worms directly into the host's intestine may lead to a higher worm recovery than previously obtained in this study, since it would circumvent the larval penetration, migration and maturation stages of the life cycle, at which points the resistance to *S.venezuelensis* infection in Edinburgh colony Wistar rats may be expressed. Thus an amount of antigenic stimulation may be produced by the transferred adults sufficient to protect animals against challenge infection to a detectable degree. Previous workers have shown that immunising rats with the adult stage of *S.ratti* gave some protection against challenge with either adults or third-stage larvae (Murrell, 1980; Bell *et al*, 1981, Nawa *et al*, 1982, and Korenaga *et al*, 1983b).

Initially it was important to discover if transferred adult *S.venezuelensis* could establish in a new host. If so, it was necessary to know the length of time adult worms of all types under study would survive in this system. This information was required to be able to give challenge infections when the

primary one had been eliminated and any non-specific inflammatory responses would have subsided.

Surgical transfer was studied in experiments 2-8 and since the protocols are related they are presented together, before an integrated treatment of the results obtained.

### 7.3.1. Materials and methods [Expts 2-8]

The techniques applicable to experiments 2-8 are described in Section 2:9, Chapter 2, page 44.

#### 7.3.1.1. Expt 2, The ability of transferred adult *S.venezuelensis* to establish in a new host

Four rats were infected with an exact dose of less than 100 adult *S.venezuelensis* worms and killed on day 4 post-infection (donor:recipient ratio 1:2).

#### 7.3.1.2. Expts 3-6, Course of infection of an exact dose of <50 adult *Strongyloides spp.* worms

The general protocol shown in Fig.7:1, page 127, was followed. The parasites used in each experiment were:-

Expt 3 - *S.venezuelensis*

Expt 4 - heterogonic strain of *S.ratti*

Expts 5/6 - homogonic strain of *S.ratti*

In experiments 3, 4 and 6, adult worms were transferred within 2 hours of the donor's death (see Expt 4, Chapter 3, page 62). In experiment 5 they were transferred later, over a period of approximately 2-3 hours.

7.3.1.3. Expt 7, Homologous challenge with adult homogonic *S.ratti*

The experimental design shown in Fig.7:2, page 129, was followed. Five rats from Gp.A, and five animals from Gp.C2, were killed on days 4 and 8 post-challenge.

7.3.1.4. Expt 8, Priming rats with adult homogonic *S.ratti* followed by homologous and heterologous challenge

The general protocol shown in Fig.7:2, page 129, was followed but in this case rats in Gp.A were challenged with either homogonic or heterogonic strain adult *S.ratti* and then killed on day 8 post-infection (Fig.7:3, page 130).

7.3.2. Results [Expts 2-8]

Adult *S.venezuelensis* seemed to be capable of establishing in a new host, since 32% of the exact dose of less than 100 adult worms was recovered on day 4 post-infection, therefore the progress with time of similar infections for the three parasites was studied.

It appeared from the results of experiments 3, 4, 5, 6 and 7 that infection with an exact dose of less than 50 adult *Strongyloides spp.* worms was falling by day 21 post-infection but within treatment variability was extreme (the coefficient of variation ranged between 30-118%). In only one case was there a significant difference in the proportion of the dose recovered over the

Fig.7:1

Eighteen rats were infected by surgical transfer with an exact dose of <50 adult *Strongyloides spp.* worms. On days 4, 14 and 21 six of the animals (randomly chosen) were killed in order to follow the course of infection.

Fig.7:1

General protocol used to find course of infection of an exact dose of <50 adult Stongyloides spp. worms

| | | TREATMENT |
|---|---|---|
| DAY 0 | 18 RATS | infected by laporotomy with an exact dose of <50 adult Strongyloides spp. worms |
| DAY 4 | 6 RATS | killed |
| DAY 14 | 6 RATS | killed |
| DAY 21 | 6 RATS | killed |

The number of eggs in utero per worm for each rat, and the number of worms present were recorded. A donor:recipient ratio of 1:6 was used and mortality was always 0%.

course of the experiment (Expt 6, Table 7:1, page 131). The high variability probably masked the effect in each of the other experiments since there was agreement in the trend in all five (Table 7:1, page 131).

From these results it was clear that rats primed with an adult infection should be challenged some time after day 21 post-infection. Challenge was therefore initiated on day 28 post-infection.

Priming rats with adult homogonic *S.ratti* (Expt 7) did protect against homologous challenge, demonstrated as a significant reduction in the number of eggs *in utero* per worm on days 4 and 8 post-challenge; and a significant reduction in the proportion of the dose recovered on day 8 compared to the relevant controls (Fig.7:4, page 133). A higher proportion of the dose was recovered from day 8 controls compared with those for day 4 ($0.01<\underline{P}<0.05$). This was clearly anomalous. If the day 8 controls were used as a measure of the unchallenged state on day 4, then challenged rats had a significantly lower worm burden on day 4 ($\underline{P}<0.001$). A significantly lower proportion of the dose was recovered from the day 8 challenge group compared with day 4, consistent with the expectation that the process of worm expulsion was cumulative over time.

Investigation into the specificity of the adult-induced immune response proved disappointing since the high variability within the data would have masked anything but gross differences in the host's response (Expt 8, Fig.7:4(ii), page 133). There did seem to be some immunity against the challenge infections since the mean proportion of the dose recovered from these groups was consistently lower than their corresponding controls though not significantly so. In addition the egg data gave some evidence for specificity in the anti-worm response. There was a reduction in this

Fig.7:2

Fourteen rats (Gps.A and C1) were infected by laporatomy with an exact dose of <50 homogonic strain of *S.ratti* worms. Four days later the priming controls (Gp.C1) were killed to check the viability of the worms used. On day 28 post-transfer the secondary controls (Gp.C2) and the primed animals (Gp.A) were infected using the surgical transfer technique with an exact dose of <50 worms of the homologous parasite (homogonic strain of *S.ratti*). On days 4 and 8 post-challenge five animals from each group (Gps.C2 and A) were killed. The proportion of the dose recovered and the number of eggs *in utero* per worm were recorded to discover when immunity (if any) was expressed.

Fig.7:2

Protocol used in Expt 7 where rats were given an homologous challenge with homogonic S.ratti adult worms

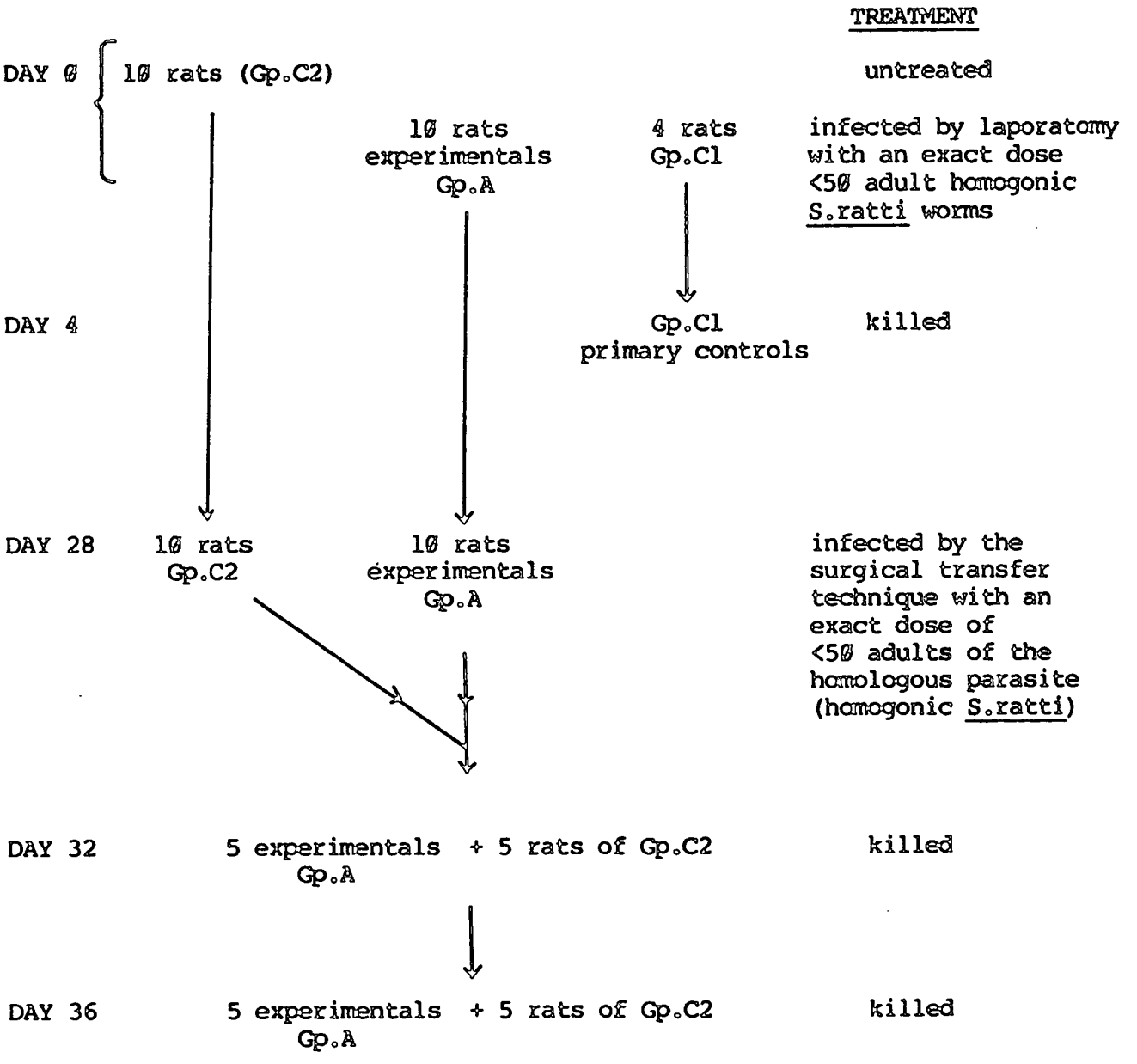


Fig.7:3

Nineteen rats (Gps.A, B and C1) were infected by surgical transfer with an exact dose of <50 adult homogonic strain *S.ratti* worms. Four days later the priming controls (Gp.C1) were killed to assess the viability of the worms used. On day 28 post-infection half the animals received a homologous challenge with an exact dose of <50 adult worms given by laporatomy (Gp.A), the remainder were given a heterologous challenge with heterogonic strain of *S.ratti* in the same manner (Gp.B). Corresponding challenge controls (Gps.C2 and C3) were also infected at the same time and all rats were killed on day 8 post-challenge. The proportion of the dose recovered and the number of eggs *in utero* per worm of the experimental groups (Gps.A and B) were compared with their relevant controls (Gps.C2 and C3 respectively) to discover if any immunity had been elicited in the primed animals.

Fig.7:3

Protocol used in challenge infections involving adult worms Expt 8

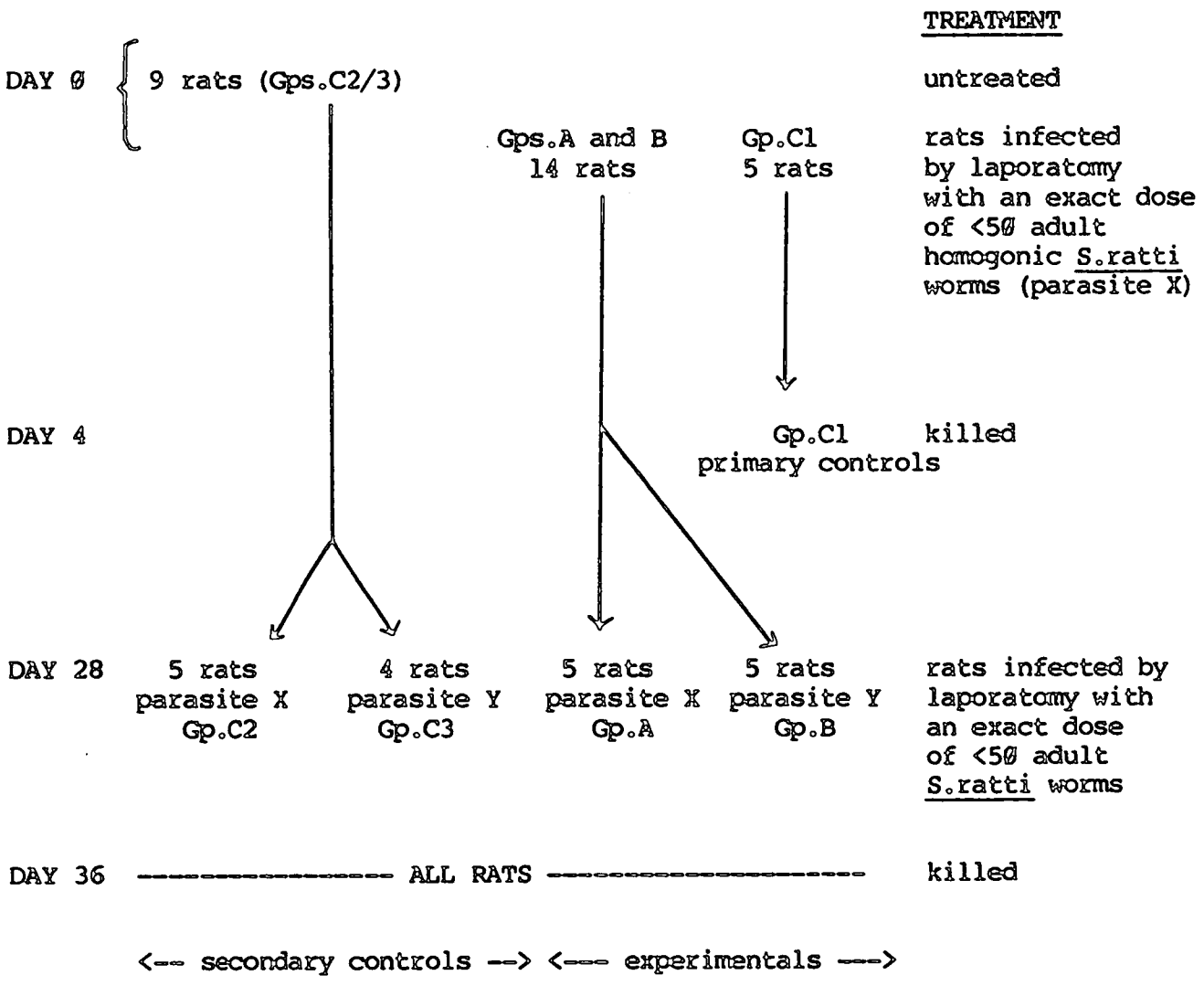


Table 7:1

Egg and worm data obtained over the course of a primary infection with an exact dose of <50 adult worms of *S.venezuelensis*, Expt 3, (a); heterogonic strain of *S.ratti*, Expt 4, (b) and homogonic strain of *S.ratti*, Expts 5 and 6 (c and d).

Table 7:1

Egg and worm data (Expts 3-6) on days 4, 14 and 21 of an adult only Strongyloides spp. infection,

a) Expt 3, S.venezuelensis

| Treatment | mean proportion of the dose recovered | n | SD |
|---|---|---|---|
| day 4 | 0.223 | 6 | 0.187 |
| day 14 | 0.127 | 6 | 0.151 |
| day 21 | 0.152 | 6 | 0.119 |
| mean exact dose | 48.6 | | |

| P values | worm data |
|---|---|
| day 4 vs 14/21 | P>0.05 |

b) Expt 4, heterogonic strain of S.ratti

| Treatment | mean proportion of the dose recovered | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|
| day 4 | 0.303 | 6 | 0.174 | 6.188 | 5 | 1.341 |
| day 14 | 0.201 | 6 | 0.063 | 3.764 | 6 | 0.797 |
| day 21 | 0.111 | 6 | 0.070 | 2.600 | 6 | 1.074 |
| mean exact dose | 48.4 | | | | | |

| P values | worm data | egg data |
|---|---|---|
| day 4 vs 14/21 | P>0.05 | |
| day 4 vs 14 | | P<0.001 |
| day 14 vs 21 | | 0.01<P<0.05 |

c) Expt 5, homogonic strain of S.ratti

| Treatment | mean proportion of the dose recovered | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|
| day 4 | 0.289 | 6 | 0.242 | 7.291 | 5 | 0.017 |
| day 14 | 0.307 | 6 | 0.154 | 6.783 | 6 | 1.312 |
| day 21 | 0.117 | 6 | 0.062 | 6.853 | 6 | 1.301 |
| mean exact dose | 48.4 | | | | | |

| P values | worm data | egg data |
|---|---|---|
| day 4 vs day 14/21 | P>0.05 | P>0.05 |

d) Expt 6, homogonic strain of S.ratti

| Treatment | mean proportion of the dose recovered | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|
| day 4 | 0.435 | 6 | 0.298 | 7.024 | 6 | 0.346 |
| day 14 | 0.293 | 6 | 0.111 | 5.348 | 6 | 1.361 |
| day 21 | 0.116 | 6 | 0.057 | 5.697 | 6 | 0.027 |
| mean exact dose | 45.9 | | | | | |

| P values | worm data | egg data |
|---|---|---|
| day 4 vs day 14 | 0.10>P>0.05 | P<0.001 |
| day 14 vs day 21 | 0.01<P<0.05 | P>0.25 |

parameter for both challenge treatments compared with their controls ($\underline{P}<0.001$, Fig.7:4(ii)b, page 133), and a significantly greater reduction after homologous challenge ($0.01<\underline{P}<0.05$, Fig.7:5, page 135).

### 7.4. Priming with a trickle infection [Expts 8 and 9]

In natural conditions the host is probably infected with small numbers of third-stage nematode larvae over an extended period of time. In the previous experiments carried out in this study, infections were the result of a single dose of larvae, admittedly much smaller than that given by most workers, but it may still have produced an abnormal host response. Therefore it was decided that the level of infection produced in rats exposed to trickle infection would be investigated, that is, repeated doses of a low number of larvae over a long period of time. Exposure to *S.venezuelensis* in this manner may lead to a cumulative worm burden, which could immunise rats against challenge infection.

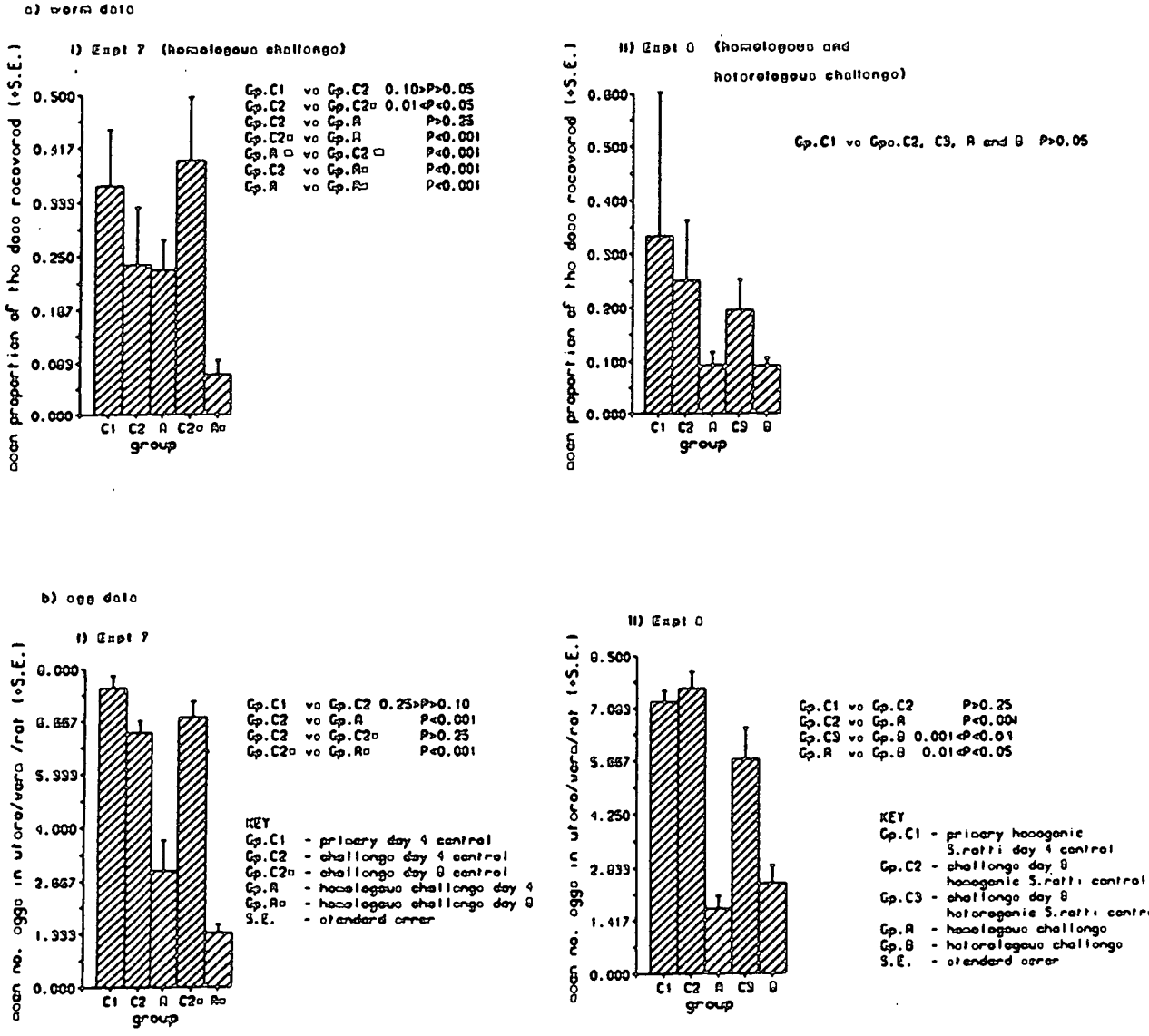
Rats, exposed to a trickle infection of 5 third-stage *S.venezuelensis* larvae per week day, were killed after various intervals of trickle infection to find out if the worm burden increased as increasing numbers of infective larvae were administered. After different times, animals from each experiment were challenged with an exact dose of less than 100 third-stage homogonic strain *S.ratti* larvae, to discover if any resistance was induced against heterologous challenge, and if so, whether it was related to the length of time the host was exposed to a trickle infection. A homologous challenge treatment was not included in the experimental protocol, since only a small proportion of a similar small dose of *S.venezuelensis* would be recovered from secondary controls and would therefore constitute an inappropriate comparison to

Fig.7:4

Rats were primed with an exact dose of <50 adult homogonic *S.ratti* worms. Twenty-eight days later the animals were given a homologous or heterologous challenge infection with a similar small dose of adult worms. In Expt 7 animals were only given a homologous challenge [Fig.7:4(i)]; whereas in Expt 8 rats were given either a homologous challenge with homogonic *S.ratti* worms or a heterologous challenge with heterogonic *S.ratti* worms [Fig.7:4(ii)]. Worm data [Fig.7:4a] and egg data [Fig.7:4b] are shown for the two experiments.

Fig.7:4

Effect of priming with adult worms of the homogonic strain of S.ratti on subsequent challenge infection, Expts 7 and 8



determine if any immune response had occurred. Experiments 9 and 10 on this topic are so similar that they are described together.

### 7.4.1. Materials and methods [Expts 9 and 10]

#### 7.4.1.1. Expt 9, Six weeks of trickle infection with *S.venezuelensis* followed by heterologous challenge with homogonic *S.ratti*

36 rats were divided into 7 groups:-

Gp.1 - 1 week of trickle infection - 5 rats, total dose planned = 25, mean dose given = 23.2

Gp.2 - 3 weeks of trickle infection - " , total dose planned = 75, mean dose given = 72.2

Gp.3 - 5 weeks of trickle infection - " , total dose planned = 125, mean dose given = 117.6

Gp.4 - 6 weeks of trickle infection - " , total dose planned = 150, mean dose given = 147.7

Gp.5 - heterologous challenge - 6 rats

Gp.6 - secondary sham *S.ratti* controls - 5 rats

Gp.7 - secondary *S.ratti* controls - 5 rats

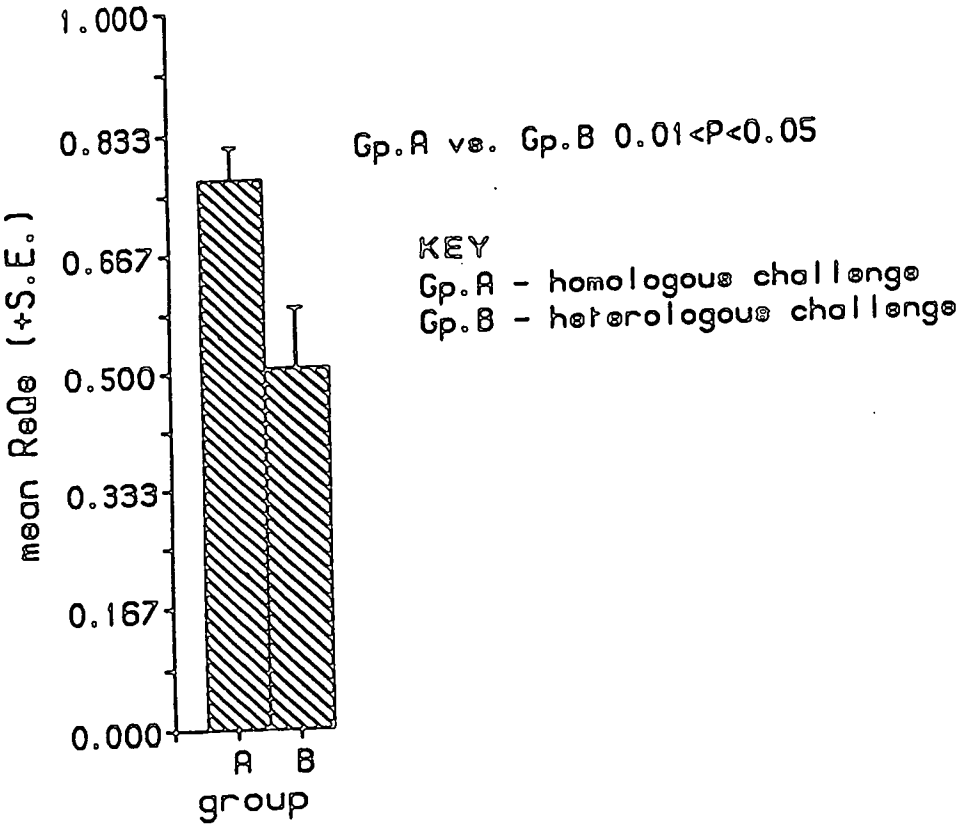
Animals which received trickle infections were injected subcutaneously without anaesthetic, every week day for the required number of weeks, with a planned number of 5 third-stage *S.venezuelensis* larvae in approximately 0.3ml distilled water using a 19G needle and a 1ml Plastipak syringe. Rats were not

Fig.7:5

The degree of protection elicited by homologous and heterologous challenge infection was compared by calculating the resistance quotients for the egg data in Expt 8. Where animals, which had been primed with an infection of adult homogonic *S.ratti* worms, were challenged 4 weeks later with a similar dose of adult worms of the homologous or heterologous parasite (heterogonic *S.ratti*). The height of the bar is directly related the amount of protection produced.

Fig.7:5

Qualitative comparison of the effect of homologous and heterologous challenge on the egg data, Expt B



injected in the same site twice to avoid complications due to the production of a local inflammatory response. Sham controls received 0.3ml saline in an identical manner over the course of trickle injections. Groups 5, 6 and 7 were then infected by skin-application of an exact dose of less than 100 third-stage homogonic strain *S.ratti* larvae (mean 97.7) three days after animals in Gp.5 had received their last trickle infection. Rats in Gps.1-3 were killed 8 days after their last injection, allowing all the larvae present to mature to the adult stage. Animals in Gps. 5-7 were killed on day 8 post-challenge.

The planned daily dose for trickle infections was corrected to account for losses during administration (see protocol above). After each injection, during the first week of both experiments, residual larvae were sought in the syringe, hypodermic needle and in the solid watch-glass from which the inoculum was drawn. However, larvae were rarely recovered from the syringe and needle washings therefore this practice was discontinued. Therefore only the number of larvae present in the watch-glass was noted. After challenge infection, in accordance with the standard "exact dose" technique (Section 2:10, Chapter 2, page 47) the number of larvae recovered from both the watch-glass and the syringe washings were found.

7.4.1.2. Expt 10, Ten weeks of trickle infection with *S.venezuelensis* followed by heterologous challenge with homogonic *S.ratti*

Experiment 10 was run in conjunction with experiment 9. Thirty-six rats were also divided into 7 groups, but trickle infections lasted longer so that :-

Gp.1 - 5 weeks of trickle infection - 5 rats, total dose planned = 125, mean dose given = 119.0

Gp.2 - 7 weeks of trickle infection - " , total dose planned = 175, mean dose given = 169.6

Gp.3 - 9 weeks of trickle infection - " , total dose planned = 225, mean dose given = 219.6

Gp.4 - 10 weeks of trickle infection - " , total dose planned = 250, mean dose given = 243.9

Gps. 5-7 were identical treatments to those described in experiment 9 (page 136) but the mean exact dose given at challenge was 95.8 larvae.

7.4.2. Results [Expts 9 and 10]

The *S.venezuelensis* worm burden did not increase in proportion to the dose administered over the course of trickle infection (Fig.7:6 and Fig.7:7, pages 139 and 140). The mean number of worms recovered were 2, 1, 3, 3 after 1, 3, 5 and 6 weeks of trickle infection respectively, in Expt 9 and in Expt 10 a maximum of 1 was recovered. Thus further trickle infection seemed to lead to a reduction in the proportion of the dose recovered but since the actual number of parasites involved at any time was low, no significance could be attached to these changes.

There may have been a reduction in the number of eggs *in utero* per worm of *S.venezuelensis* adults recovered over the course of infection, but since this observation was based on few replicates (i.e. worms) per treatment, no real judgements could be made.

Priming rats with a trickle infection of 5 infective *S.venezuelensis* larvae per week day for 6 or 10 weeks failed to protect animals against heterologous

challenge infection with an exact dose of less than 100 third-stage homogonic *S.ratti* larvae, since there was no reduction in the proportion of the dose recovered, or in the number of eggs *in utero* per worm of the challenged group compared with secondary controls (Figs 7:6 and 7:7, pages 139 and 140).

Adult *S.venezuelensis* were recovered from the heterologous challenge group in both experiments 9 and 10 (shown separately as Gp.8 in Figs.7:6 and 7:7, pages 139 and 140). However the number of *S.venezuelensis* adults was very small, and clearly quite insufficient to compete with *S.ratti* for resources.

### 7.5. Priming immature rats with *S.venezuelensis* [Expt 11]

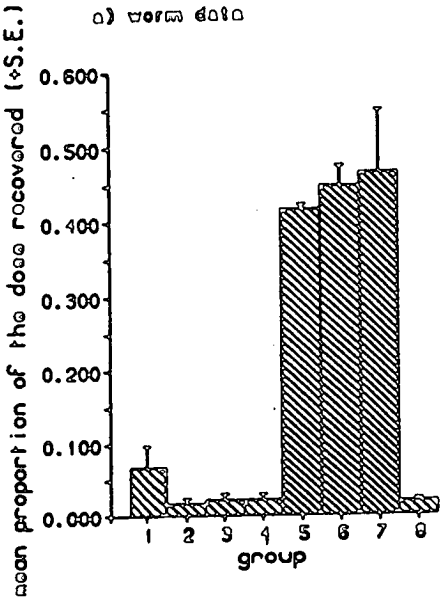
Experiments by previous workers have suggested that young animals have a reduced resistance to a number of parasites compared to their adult counterparts. For example, young rats are more susceptible to infection with *N.brasiliensis* (Jarrett *et al*, 1966, 1968, 1969), *Plasmodium berghei* (Smalley, 1975), and *Fasciola hepatica* (Hayes *et al*,1974). Similarly, calves are more susceptible to *Cysticercus bovis* (Soulsby, 1963). These examples are consistent with general experience that some component(s) of the immune system is(are) not fully functional until the host reaches a certain age. In the present study it was found that young rats were more susceptible to infection with *S.venezuelensis* compared with adults (results of Expts 4-6, Chapter 6, page 115). Therefore it was decided that the effect of priming three-week old animals with an exact dose of less than 200 infective *S.venezuelensis* larvae, followed by a challenge infection nine weeks later, would be investigated. If some protection was elicited by this regime, it might have offered a framework to study cross resistance with the *S.ratti* varieties.

Fig.7:6

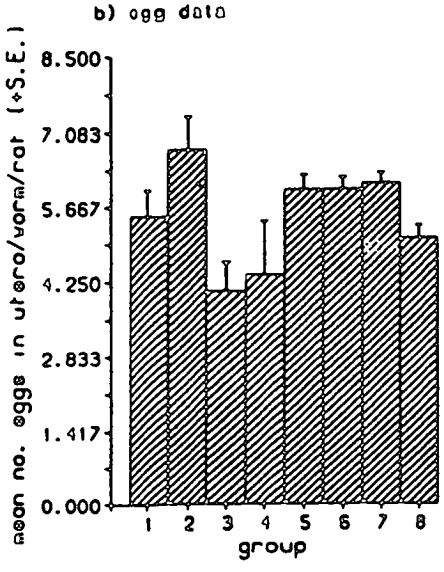
The effect of 5 weeks of trickle infection with *S.venezuelensis* on subsequent challenge with homogonic *S.ratti* was investigated to find if any immunity against the priming agent had been elicited. The doses given to rats over the course of trickle infection are shown in the figure.

Fig.7:6

Effect of 6 weeks of trickle infection with S.venezuelensis on subsequent heterologous challenge with the homogonic strain of S.ratti, Expt 9




Gp.6 vs Gps.7 and 8 P>0.25




Gp.5 vs. Gps.6/7 P>0.25

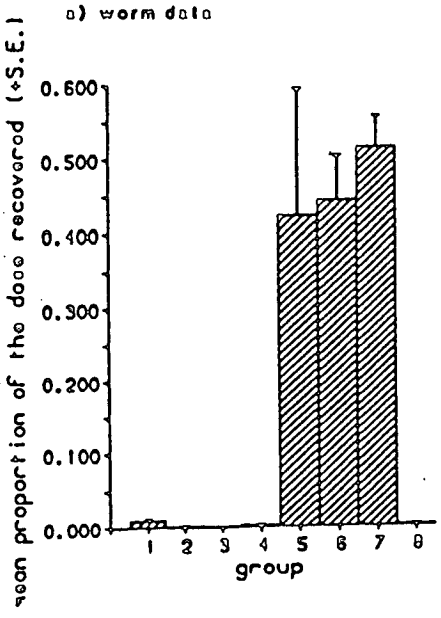
KEY
Gp.1 - 1 week of trickle infection (mean dose given = 23.2)
Gp.2 - 3 weeks of trickle infection (mean dose given = 72.2)
Gp.3 - 5 weeks of trickle infection (mean dose given = 117.6)
Gp.4 - 6 weeks of trickle infection (mean dose given = 147.7)
Gp.5 - heterologous challenge with homogenic S.ratti
Gp.6 - challenge homogenic S.ratti controls
Gp.7 - challenge homogenic S.ratti chem controls
Gp.8 - S.venezuelensis worms recovered from Gp.5 rats
S.E. - standard error

Fig.7:7

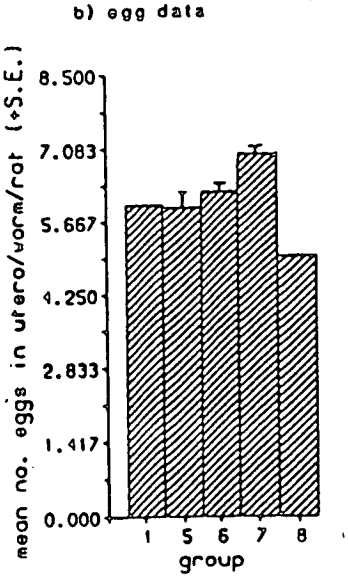
Rats were given a trickle infection with *S.venezuelensis* for 9 weeks and then challenged with homogonic *S.ratti* to discover if any immunity against the priming agent had been produced. The doses administered to animals over the course of trickle infection are shown in the figure.

Fig.7:7

Effect of 10 weeks of trickle infection with S.venezuelensis on subsequent heterologous challenge with the homogonic strain of S.ratti, Expt 10




Gp.6 vs. Gps.8/7 P>0.25




Gp.6 vs. Gp.7 $0.01<P<0.05$
Gp.5 vs. Gp.7 $0.001<P<0.01$
Gp.5 vs. Gp.6 $P>0.25$

KEY
Gp.1 - 5 weeks of trickle infection (mean dose given = 119.01
Gp.2 - 7 weeks of trickle infection (mean dose given = 169.61
Gp.3 - 9 weeks of trickle infection (mean dose given = 219.61
Gp.4 - 10 weeks of trickle infection (mean dose given = 243.91
Gp.5 - heterologous challenge with homogonic S.ratti
Gp.6 - challenge homogonic S.ratti controls
Gp.7 - challenge homogonic S.ratti sham controls
Gp.8 - S.venezuelensis worms recovered from Gp.5 rats
S.E. - standard error

### 7.5.1. Materials and methods [Expt 11]

The protocol shown in Fig.7:8 (page 142), was followed.

### 7.5.2. Results [Expt 11]

Priming immature rats with *S.venezuelensis* elicited a significant protective response against both homologous and heterologous challenge, demonstrated as a significant reduction in the number of eggs *in utero* per worm for all three treatments compared with their corresponding controls ($P<0.001$, Table 7:2, page 143). The host's anti-worm response was also demonstrated as a significant reduction in the proportion of the dose recovered for the heterologous challenge treatments compared with controls ($P<0.01$, Table 7:2, page 143). A similar reduction was not obtained after homologous challenge due to the previously demonstrated age-related resistance to *S.venezuelensis* infection in the Edinburgh colony Wistar rats (results of Expts 4-6, page 114) therefore only a low proportion of the dose was recovered from secondary adult controls making it impossible to determine if a reduction had occurred in challenged animals. This age-related resistance was also demonstrated in this experiment since a significantly lower proportion of the dose, and a significantly lower number of eggs *in utero* per worm, was recovered from secondary, compared with primary, controls (Table 7:2, page 143).

Quantitative comparison of the level of immunity elicited by each parasite, assessed as the relative reduction in the number of eggs *in utero* per worm revealed significant differences. Maximum immunity was expressed against homologous challenge, followed by heterologous challenge using heterogonic *S.ratti*, with the minimum produced by heterologous challenge with homogonic

Fig.7:8

Twenty-nine three-week old Wistar rats (Gps.A, B, D and C1) were infected by skin-application with an exact dose of <200 third-stage *S.venezuelensis* larvae. On day 8 post-infection five of the animals (priming controls, Gp.C1) were killed to check the viability of the larvae used. Nine weeks later animals in Gps.A, B and D were challenged with an exact dose of either the homologous (Gp.A), or heterologous parasite (homogonic strain of *S.ratti*, Gp.B; heterogonic strain of *S.ratti*, Gp.D) in the same manner used at priming and corresponding challenge controls were infected at the same time (Gp.C2 for Gp.A; Gp.C3 for Gp.B; and Gp.C4 for Gp.D). Eight days later all the rats were killed and the proportion of the dose recovered and the number of eggs *in utero* per worm recorded for each rat. The effectiveness of the different types of challenge treatments were found by calculating resistance quotients for the egg and worm data of each.

Fig.7:8

Protocol used to immunise immature rats against heterologous challenge.

Expt 11

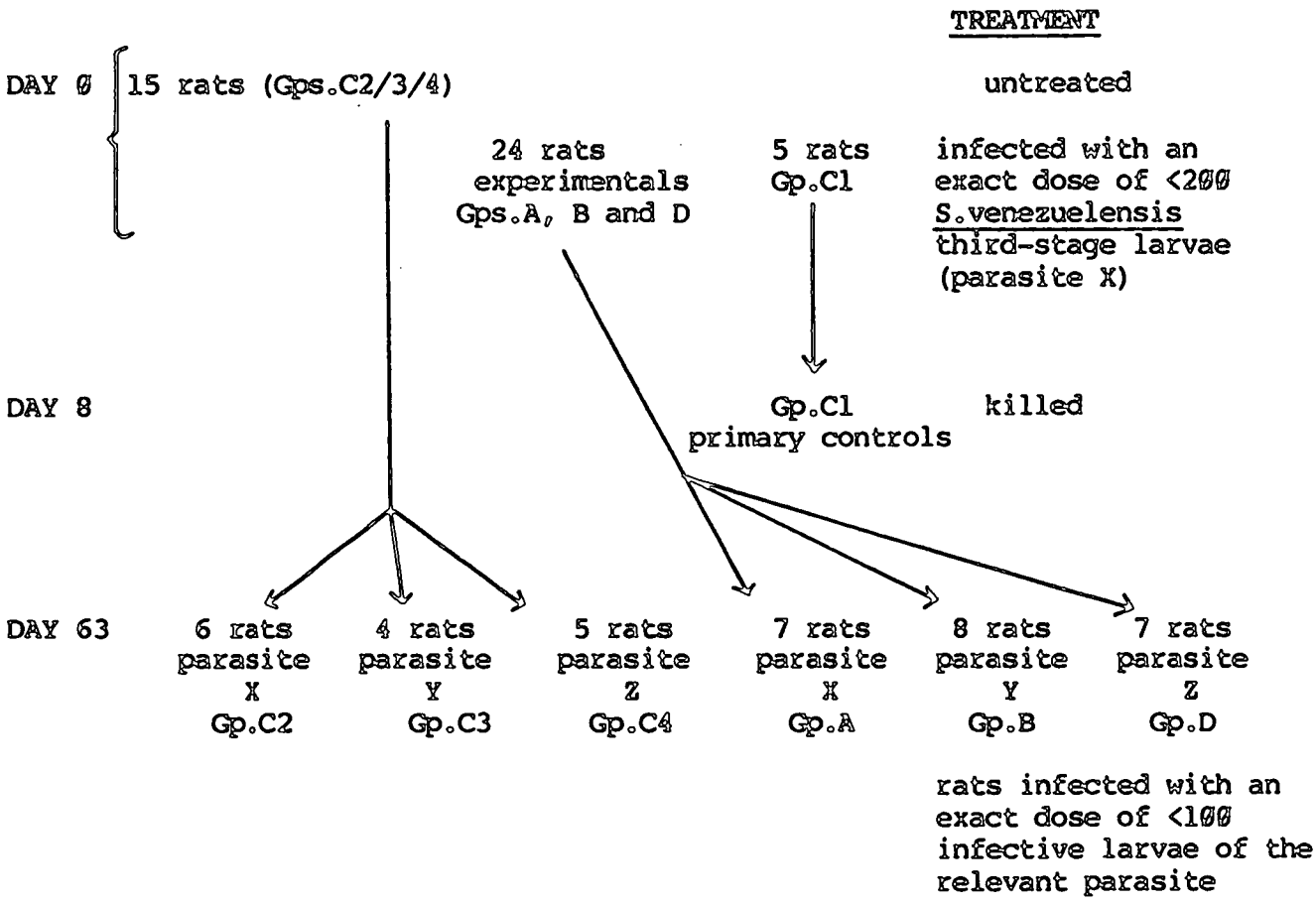


Table 7:2

Egg and worm data obtained on day 8 post-challenge from rats which were primed with an exact dose of <200 third-stage *S.venezuelensis* larvae when they were 3 weeks old and then challenged with an exact dose of <100 infective larvae of the relevant parasite 9 weeks later. *S.venezuelensis* adults were recovered from rats given a heterologous challenge (Gps.B and D) and their results are shown separately as Gps.B2 and D2.

Table 7:2

Homologous vs heterologous challenge: S.venezuelensis priming immature rats,

Expt 11

a) Worm data

| Treatment | Group | S.venezuelensis mean prop. of the dose recovered | n | SD | S.rotti mean prop. of the dose recovered | n | SD |
|---|---|---|---|---|---|---|---|
| homologous challenge | Gp.A | 0.015 | 7 | 0.02 | | | |
| heterologous challenge homogenic strain of S.rotti | Gp.B | | | | 0.351 | 8 | 0.08 |
| heterologous challenge homogenic strain of S.rotti, S.venezuelensis worms | Gp.B2 | 0.010 | 8 | 0.02 | | | |
| heterologous challenge heterogenic strain of S.rotti | Gp.D | | | | 0.162 | 7 | 0.04 |
| heterologous challenge heterogenic strain of S.rotti, S.venezuelensis worms | Gp.D2 | 0.004 | 7 | 0.01 | | | |
| priming control | Gp.C1 | 0.036 | 5 | 0.04 | | | |
| challenge control S.venezuelensis | Gp.C2 | 0.023 | 6 | 0.04 | | | |
| challenge control homogenic S.rotti | Gp.C3 | | | | 0.477 | 4 | 0.04 |
| challenge control heterogenic S.rotti | Gp.C4 | | | | 0.466 | 5 | 0.12 |
| mean exact dose | priming challenge | S.venezuelensis | | | 191.04 | | |
| | | S.venezuelensis | | | 94.33 | | |
| | | homogenic S.rotti | | | 98.00 | | |
| | | heterogenic S.rotti | | | 94.00 | | |

b) Egg data

| Treatment | Group | S.venezuelensis mean no. of eggs in utero/worm/rat | n | SD | S.rotti mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|
| homologous challenge | Gp.A | 1.750 | 4 | 1.26 | | | |
| heterologous challenge homogenic strain of S.rotti | Gp.B | | | | 4.712 | 8 | 0.90 |
| heterologous challenge homogenic strain of S.rotti, S.venezuelensis worms | Gp.B2 | 1.300 | 5 | 1.32 | | | |
| heterologous challenge heterogenic strain of S.rotti | Gp.D | | | | 2.202 | 7 | 0.57 |
| heterologous challenge heterogenic strain of S.rotti, S.venezuelensis worms | Gp.D2 | 1.500 | 2 | 0.71 | | | |
| priming controls | Gp.C1 | 7.861 | 5 | 0.52 | | | |
| challenge controls S.venezuelensis | Gp.C2 | 6.125 | 2 | 1.59 | | | |
| challenge controls homogenic S.rotti | Gp.C3 | | | | 6.583 | 4 | 0.17 |
| challenge controls heterogenic S.rotti | Gp.C4 | | | | 5.467 | 5 | 0.30 |

| P values | worm data | egg data |
|---|---|---|
| Gp.C2 vs B/D2/E2 | P>0.25 | P<0.001 |
| Gp.C3 vs B | 0.001<P<0.01 | P<0.001 |
| Gp.C4 vs D | P<0.001 | P<0.001 |
| Gp.C3 vs C4 | P>0.25 | 0.01<P<0.05 |
| Gp.C1 vs C3/C4 | P>0.25 | |
| Gp.C1 vs D2/E2 | P<0.001 | P<0.001 |

*S.ratti* (Fig.7:9, page 145).

A similar comparison was carried out using the worm data. Initially all three treatments were included in the analysis, but because there was a much higher variability in the homologous challenge treatment, it was omitted from statistical analysis of the data. The outcome of this comparison was consistent with that already described for the egg data. A significantly greater reduction in the proportion of the dose recovered was produced after heterologous challenge with heterogonic *S.ratti*, compared with that induced by homogonic *S.ratti* challenge infection ($P<0.001$, Fig.7:9, page 145).

Adult *S.venezuelensis* were recovered from the heterologous challenge treatments (shown as Gps.B2 and D2 in Table 7:2, page 143) demonstrating that the host's immune response against the primary infection was not fully functional in expelling all those worms from the intestine.

Data on the longitudinal distribution of adult *Strongyloides spp.* recovered from experiments 2-11, are shown in Tables 6:2(i)-(x), Appendix 6, page 326. Posterior migration of worms in the challenge treatments seemed to occur in Expts 7 and 8; for, compared with controls, adults were recovered further down the intestine on day 8 post-challenge in experiment 7, and after heterologous challenge in experiment 8 (Tables 6:2(vi)-(vii), Appendix 6, page 327 ). However this was based on so few parasites that no real significance could be attached to the observation. The distribution of adult *S.venezuelensis* in Wistar rats seemed to be more restricted than that of *S.ratti*, since worms of the former species were only recovered from the first three sections of the intestine. For example, worms recovered from primary controls in experiment 11 were found exclusively from these sections, and this observation was based on the relative position of 83 adults (Table 6:2(x), Appendix 6, page 329).

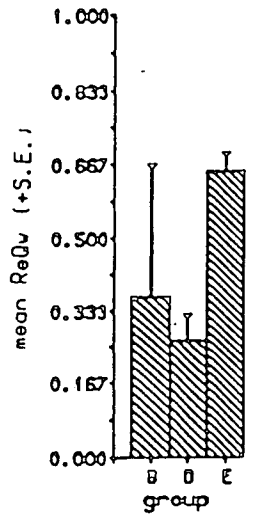
Fig.7:9

Animals, which had been primed with an exact dose of <200 infective *S.venezuelensis* larvae when they were 3 weeks old, were challenged 9 weeks later with an exact dose of <100 third-stage larvae of either the homologous or heterologous parasite. The amount of protection induced was assessed by calculating the resistance quotients for the three treatments. The amount of resistance induced by the three treatments can be seen clearly since the height of the bar in the figure is directly related to the degree of protection induced.

Fig.7:9

Comparison of the amount of protection induced in rats after homologous and heterologous challenge infection: S.venezuelensis priming animals, Expt II

a) worm data



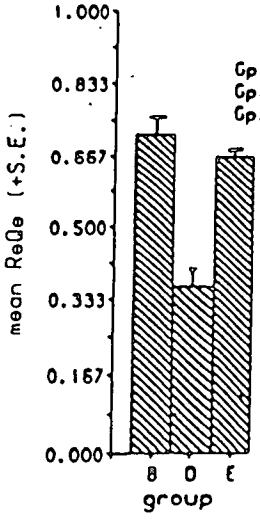

Gp.B vs Gps.D and E P>0.05

If only Gps.D and E are used in the analysis then-

Gp.B vs Gp.E P<0.001

KEY
Gp.B - homologous challenge
Gp.D - heterologous challenge, homogenic S.ratti
Gp.E - heterologous challenge, heterogenic S.ratti
S.E. - standard error

b) egg data




Gp.B vs Gp.D P<0.001
Gp.D vs Gp.E P<0.001
Gp.B vs Gp.E 0.01<P<0.05

Thus this would seem to be a real feature of the biology of *S.venezuelensis*, and the data from these experiments give credence to others carried out in the present study, in which a similar distribution was detected, but with too few worms to provide concrete evidence (Expts 9 and 10, Tables 6:2(viii) and (ix), Appendix 6, page 328). Adult *S.ratti*, on the other hand, are recovered as far as sections 5 and 6 of the intestine (Expts 9, 10 and 11, Tables 6:2(viii)-(x), Appendix 6, page 328). A number of common experimental techniques were used throughout this section and they can be found in the relevant section of Chapter 2, shown below:-

a) infection by skin-application and subcutaneous injection, Section 2:10, page 47.

b) method of worm counting and counting the number of eggs *in utero* per worm, Section 2:10, page 50.

c) statistical analysis of results, Section 2:11, page 51.

## 7.6. Summary

a) Priming with a single dose of 10,000 heat-killed homogonic *S.ratti* larvae failed to protect rats against homologous challenge infection with an exact dose of less than 100 infective larvae.

b) Transferred *Strongyloides spp.* adults were capable of establishing in a new host. The course of a primary infection with an exact dose of less than 50 transferred worms lasted about 21 days. An exact dose of <50 adult homogonic *S.ratti* worms immunised animals against homologous challenge with a similar small adult infection dose, which was expressed as a reduction in the number of eggs in utero per worm, and a reduction in the proportion of the dose recovered. There appeared to be some specificity in the immune response since homologous challenge with homogonic *S.ratti* produced a significantly greater reduction in the number of eggs *in utero* per worm compared with heterologous challenge with heterogonic *S.ratti.* Unfortunately all experiments displayed high variability within the treatments, so clearly there were unknown facets of the experimental procedure which were insufficiently under control.

c) Trickle infection for 6 or 10 weeks with 5 *S.venezuelensis* infective larvae per week day did not lead to the recovery of a large number of adults, nor did it protect rats against subsequent heterologous infection with an exact dose of less than 100 homogonic *S.ratti* larvae.

d) Immunising immature rats with an exact dose of less than 200 *S.venezuelensis* larvae induced a protective immune response against subsequent challenge infection with an exact dose of <100 *Strongyloides spp.* infective larvae. This appeared as a reduction in the number of eggs *in utero*

per worm (all treatments) and a reduction in the proportion of the dose recovered (heterologous treatments only). Quantitative comparison of the level of immunity produced, as assessed by the relative reduction in the number of eggs *in utero* per worm, suggested there was a graded immune response. Using this criterion, homologous challenge produced the maximium amount of immunity, followed, in order, by heterologous challenge with heterogonic and then homogonic *S.ratti*. If the comparison was carried out using worm data, there was a significantly greater reduction in the proportion of the dose recovered after challenge with heterogonic *S.ratti*, compared with the homogonic strain of the species. The homologous challenge treatment was not included in this comparison, since the very high variability in this particular treatment rendered a pooled estimate of error variance across all treatments suspect. With this previso, the data obtained by counting worms were consistent with those from eggs *in utero*.

CHAPTER 8

# ADOPTIVE TRANSFER OF IMMUNITY AGAINST *STRONGYLOIDES SPP.*

## 8.1. Introduction

The experiments in Chapter 5 have shown that prior infection with an exact dose of less than 100 *S.ratti* larvae induced a significant resistance to challenge infection, and that there was a quantitative difference in the level of immunity elicited depending on the strain of *S.ratti* used to prime rats (see results of Expts 4, 5, 6 and 7, Chapter 5, page 99).

The underlying asymmetry detected by these experiments could have been related:-

a) to antigen differences between the strains (see discussion)

or b) a quantitative or qualitative difference in the non-specific detrimental effect that both strains had on the intestinal enviroment during primary infection.

In order to decide between these alternatives the following adoptive transfer experiments were carried out using inbred rats of the PVG strain. These experiments had four main aims:-

i) to find out if resistance against *Strongyloides spp.* could be transferred from immunised to naive rats with mesenteric lymph node cells, and if so, when it was expressed.

ii) to find out if such cells primed against an exact dose of <100 *Strongyloides spp* larvae could protect against a similar small challenge

infection.

iii) to discover whether transferred cells would protect against challenge infection with the heterologous parasite.

iv) to see whether there was any asymmetry in (i), (ii) and (iii) dependent on which strain was used for priming and, if so, whether the pattern mimicked the one already revealed by earlier experiments (see above).

The results obtained suggested that infection of rats with a large inoculum of third-stage *S.venezuelensis* larvae led to a higher proportion of the dose recovered than previously, using small exact doses (results of Expt 3, shown in Table 5:1(iii), Appendix 5, page 319). Thus it may be possible to immunise rats against the parasite using large doses so that their mesenteric lymph node cells could be used in reciprocal cross-immunity studies along with the two strains of *S.ratti*.

The cell transfer method used throughout experiments was similar to that of Moqbel and Wakelin (1981), who demonstrated that transfer of immune mesenteric lymph node cells taken late post-primary infection, from rats infected with 1000 third-stage *S.ratti* larvae, protected against homologous challenge. Ideally $2.5 \times 10^8$ cells were administered per rat, but this was not always possible, therefore the actual number of cells given is shown for each experiment.

The experiments described in this chapter are divided into three sections which contain related studies.

## 8.2. Protection induced by homologous challenge assessed on day 8 post-challenge

It had already been found that rats primed against *S.ratti* and challenged 28 days later demonstrated a significant resistance to re-infection on day 8 post-challenge (Chapter 5, page 93). In a similar experimental framework, could animals, primed with immune mesenteric lymph node cells and assayed on day 8 post-challenge, be shown to be resistant to infection?

### 8.2.1. Materials and methods

#### 8.2.1.1. Expts 1 and 2, Comparison of the level of immunity induced in rats after a secondary infection and homologous challenge after adoptive transfer

The protocol used in Expts 1 and 2 is summarised in Fig.8:1, page 152. In experiment 1, $5x10^7$ mesenteric lymph node cells were administered to each rat, in experiment 2 the number was increased to $2x10^8$cells per rat. Ideally the animals receiving immune cells should have been given $2.5x10^8$ cells each but the yield from the cell donors was too small, therefore the lower number had to suffice.

#### 8.2.1.2. Expt 3, The effect of increasing the infection dose in the rats which supply the immune cells

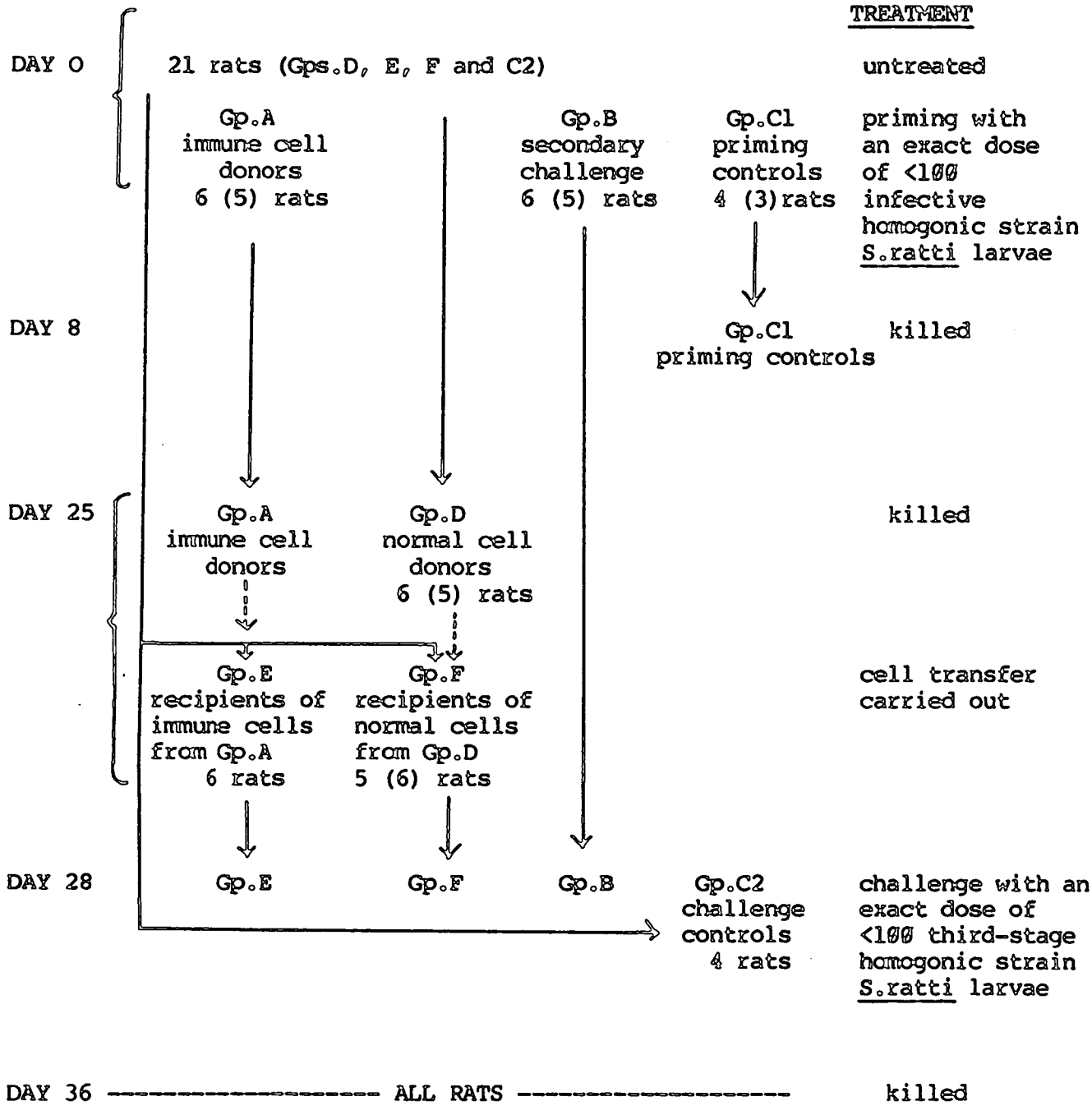
The protocol used in Expts 1 and 2 was modified since the secondary infection and the "normal" cell treatments, Gps. "B", "D" and "F", were omitted. Cell donors were infected with a mean of 1000 or 4000 infective homogonic *S.ratti* larvae instead of a small "exact" dose. The procedure followed is

Fig.8:1

On day 0 rats in groups A, B and C1 were infected by skin-application with an exact dose of <100 third-stage *S.ratti* larvae of the homogonic strain. Eight days later the priming controls (Gp.C1) were killed to give an estimate of the viability of larvae used at priming. The adoptive transfer of mesenteric lymph node cells was carried out on day 25 post-infection, animals in Gp.E received immune cells, donated by rats in Gp.A, whereas rats in Gp.F received normal cells prepared from rats in Gp.D. Three days later rats in Gps. B, E, F and C2 (challenge controls) were infected by skin-application with an exact dose of <100 homogonic strain *S.ratti* larvae. On day 8 post-challenge all rats were killed and the efficacy of the immune cells was found by comparing the proportion of the dose recovered, and the number of eggs *in utero* per worm, from Gp.E (recipients of immune cells) and secondary controls (Gp.C2). Results from Gp.D (which received normal cells) showed whether the technique used had any effect on the parasite burden and results from Gp.B (secondary infection) demonstrated how effective priming with a full infection compared with priming with immune cells only. The numbers in brackets refer to the number of animals used in Expt 2.

Fig.8:1

Protocol used in Expts 1 and 2




KEY

_____ indicates progress of the same animals

------- indicates transfer of mesenteric lymph node cells

shown in Fig.8:2, page 154

When animals were killed at the relevant time the number of adults present in their intestines and the number of eggs *in utero* per worm was counted.

### 8.2.2. Results

From the results of experiments 1 and 2 (Fig.8:3, page 155) it was clear that little, or no, immunity was adoptively transferred to rats by the procedure under test (Gp.F vs Gp.E) whereas a highly significant resistance was obtained after secondary infection (Gp.C2 vs B). In both experiments the low proportion of the challenging dose recovered from rats which had a primary infection (Gp.B) compared with their controls (Gp.C2) was quite emphatic ($P<0.001$), as also was the reduction in the number of eggs *in utero* ($P<0.001$).

In experiment 1 there was a slight reduction in the proportion of the dose recovered from rats which received immune cells compared with controls; however, a corresponding reduction was not obtained in experiment 2, where more immune cells were given to each rat, and where a greater level of protection might therefore be expected. There was a reduction in the number of eggs *in utero* per worm after challenge, for both experiments, compared to controls (Gp.E vs Gp.C2, $0.01<\underline{P}<0.05$, in Expt 1; $\underline{P}<0.001$, in Expt 2, Fig.8:3(b), page 155, suggesting that some anti-worm response had occurred. A corresponding reduction in the number of eggs *in utero* per worm was not obtained in rats which received normal cells, inferring that the transfer technique was not responsible for this effect.

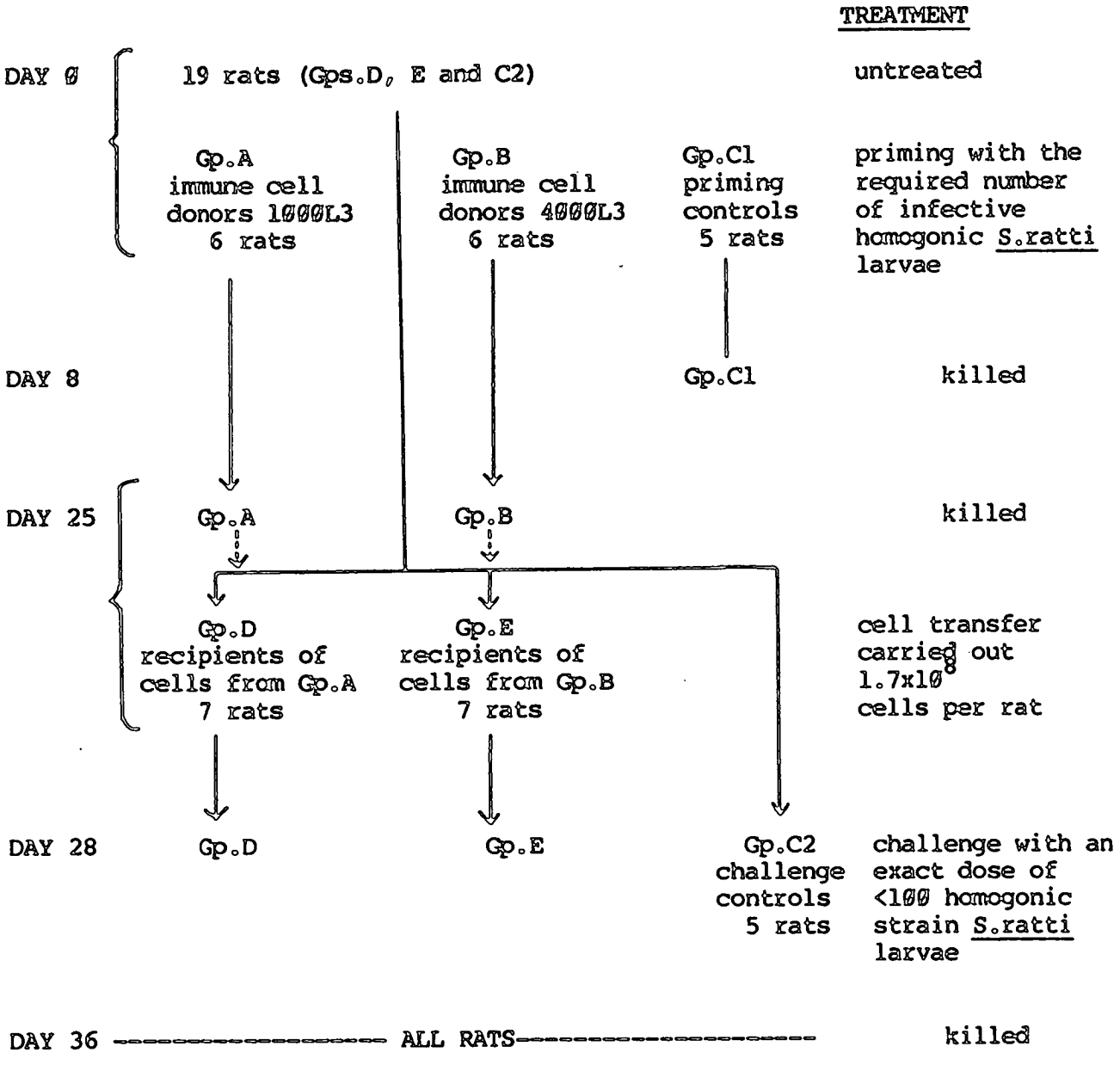
There was no obvious difference in the distribution of adults recovered from secondary controls, challenged animals and donor rats (killed on day 25

Fig.8:2

Rats in groups A, B and C1 were infected by skin-application with third-stage *S.ratti* larvae of the homogonic strain; Gp.C1 (priming controls) received an exact dose of <100 larvae, 1000 larvae were administered to rats in Gp. A, whilst Gp.B animals received 4000 larvae. The priming controls were killed on day 8 post-infection to check the viability of the larvae used at priming and 17 days later adoptive transfer of immune mesenteric lymph node cells to rats in Gps. D and E was carried out. Three days later these animals and challenge controls (Gp.C2) were infected by skin-application with an exact dose of less than 100 infective *S.ratti* larvae of the homogonic strain. All rats were killed on day 8 post-challenge and comparison of the proportion of the dose recovered and the number of eggs *in utero* per worm from rats in Gps. D and E showed whether increasing the infection dose in the cell donors affected the level of immunity transferred.

Fig.8:2

Protocol used in Expt 3




KEY

_____ indicates progress of the same animals

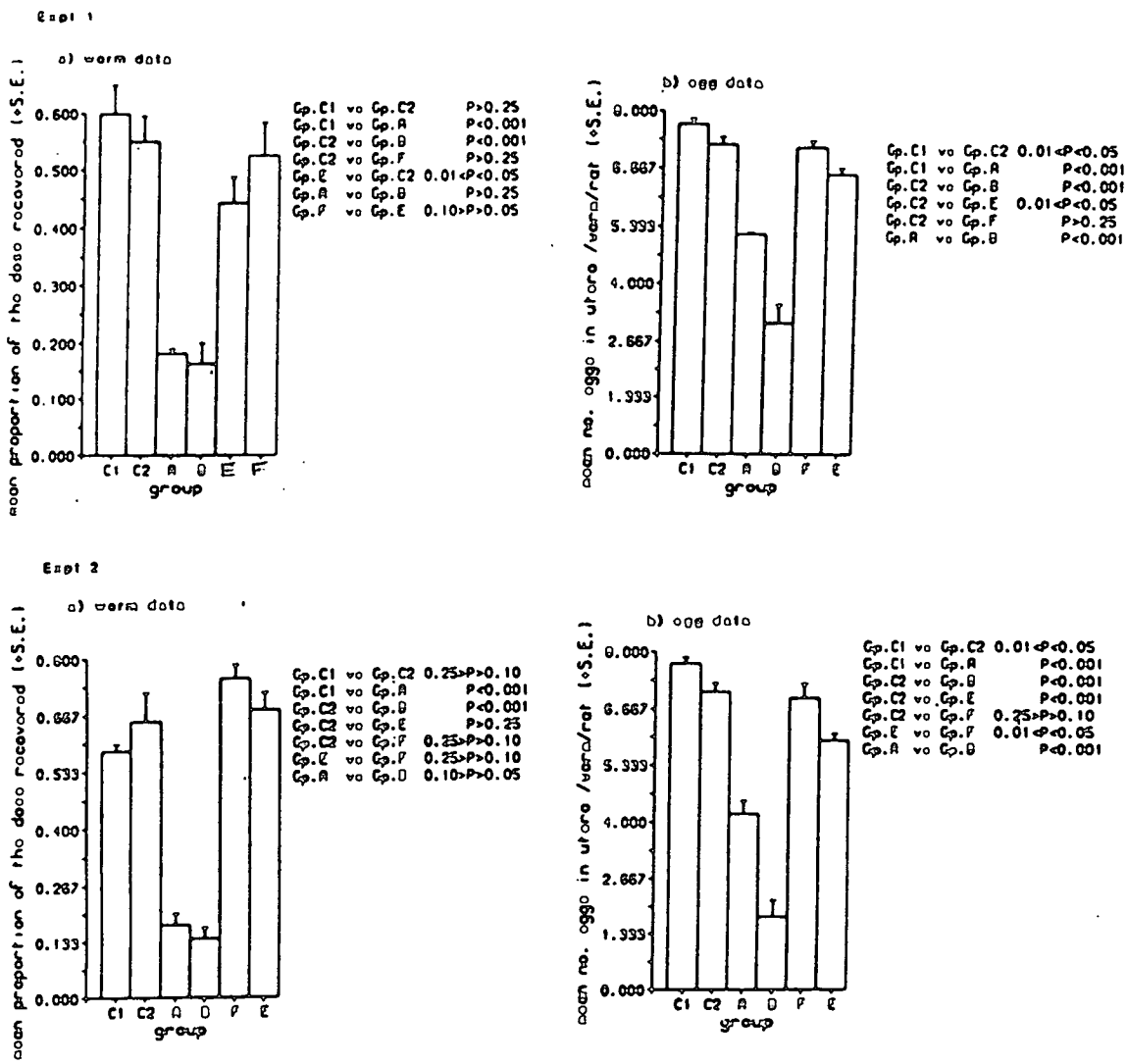
------- indicates transfer of mesenteric lymph node cells

Fig.8:3

The effect of priming rats with either a full infection (Gp.B), or with immune mesenteric lymph node cells (Gp.F) (- obtained from donors infected with an exact dose of <100 homogonic *S.ratti* infective larvae), on subsequent homologous challenge was investigated. Animals were killed on day 8 of the challenge infection.

Fig.8:3

Priming of cell donors with homogonic strain of S.ratti: autopsy of recipients 8 days after challenge infection, Expts 1 and 2




KEY
Gp.C1 - primary control
Gp.C2 - challenge control
Gp.A - day 25 primary infection (immune cell donor)
Gp.B - secondary infection
Gp.E - recipients of immune mesenteric lymph node cells
Gp.F - recipients of normal mesenteric lymph node cells
S.E. - standard error

of a primary infection). There was a possible posterior migration of worms from the secondary infection group, but this observation was based on the relative position of a few worms (Tables 7:2(i) and (ii), Appendix 7, page 335).

Increasing the level of infection in the cell donors did not have a dramatic effect on the results obtained. The cells did transfer some immunity since there was a significant reduction in the number of eggs *in utero* per worm compared with controls (Gp.D/E vs Gp.C2, $P<0.001$), but a corresponding reduction in the proportion of the dose recovered did not occur (Fig.8:4, page 158), reflecting the results of experiments 1 and 2. There was no dose response effect demonstrated in the egg data since there was no significant difference in this parameter between the challenged groups which received cells from rats infected with either 1000, or 4000 homogonic *S.ratti* infective larvae (Gp.D vs Gp.E, $P>0.25$, Fig 8:4b, page 158). There did not seem to be any posterior migration of adults in any treatment, except perhaps the cell donors, killed on day 25 post-primary infection (Table 7:2(iii), Appendix 7, page 336) but again this observation was based on the relative position of only a few worms.

Since very little immunity was transferred by this protocol, the effect of killing rats later post-challenge was investigated.

### 8.3. Resistance induced by homologous challenge assessed on day 16 post-challenge

Although some protection was demonstrated in Expts 1-3 following the transfer of primed cells it was too slight at day 8 post-challenge to allow fine distinctions to be drawn between types of parasite. It was possible, however, that the response would be more emphatic at day 16 of the infection;

therefore the following experiments were designed to examine this question. The same general protocol was used throughout experiments 4-7 (Fig.8:5, page 160). Rats, at transfer, received cells which had been primed either against an exact dose of less than 100 *S.ratti* infective larvae or against an estimated mean dose of 2000. Each recipient should have received a standard dose of $2.5x10^8$ immune mesenteric lymph node cells, but in some cases where the yield from cell donors was too small a lower dose was administered.

### 8.3.1. Materials and methods

#### 8.3.1.1. Expts 4 and 5

The homogonic strain of *S.ratti* was used to prime cell donors and $2.5x10^8$ cells were given to each recipient.

#### 8.3.1.2. Expts 6 and 7

Donors were primed against the heterogonic strain of *S.ratti* and $2x10^8$ cells were administered to every rat in the relevant treatment in Expt 6, and $1.77x10^8$ cells in Expt 7.

When rats were killed at the appointed time the number of adults present, their distribution along the intestine and the number of eggs *in utero* per worm was found.

### 8.3.2. Results

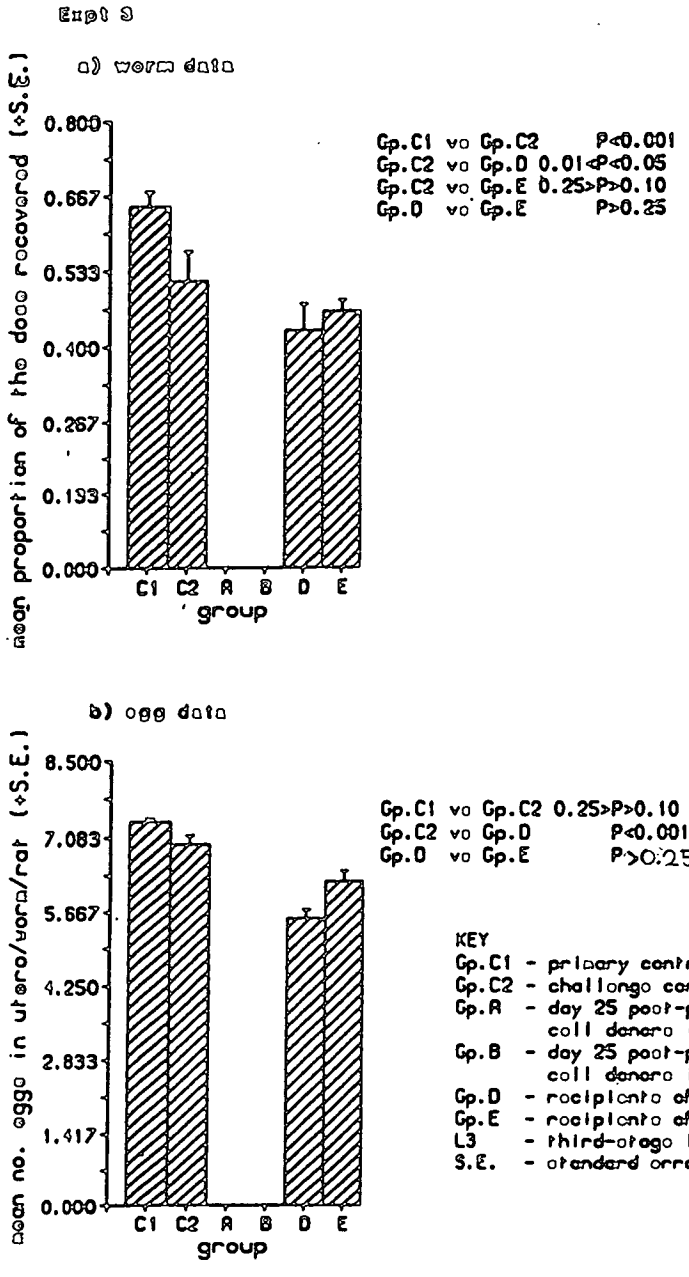
Homologous challenge with either strain of *S.ratti*, at both infection levels,

Fig.8:4

The effect of increasing the infection dose in the cell donors on the amount of protection transferred to recipients was investigated. The cell donors (Gps.A and B) were infected with either 1000 or 4000 third-stage homogonic *S.ratti* larvae (respectively) . The recipients (Gps.D and E) were challenged with an exact dose of <100 third-stage larvae of the homologous parasite and killed on day 8 of the challenge infection.

Fig.8:4

Effect of increasing homogonic S.ratti larval dose used to prime cell donors: autopsy of recipients 8 days after challenge infection, Expt 3



induced a significant resistance to re-infection expressed as a reduction in the proportion of the dose recovered (Gp.D/E vs Gp.C3, $P<0.001$) and a reduction in the number of eggs *in utero* ($P<0.001$, Table 8:1, page 161).

If the homogonic strain was used to prime the immune cells transferred to rats, then those from donors infected with 2000 third-stage larvae transferred a greater degree of immunity than those from rats infected with <100 parasites. This was demonstrated as a greater reduction in the proportion of the dose recovered (Gp.D vs Gp.E, $P<0.001$), as well as in the number of eggs *in utero* per worm ($P<0.001$, Table 8:1, page 161). However if the heterogonic strain was used to prime cells a dose-response effect of similar intensity was absent (Gp.D vs Gp.E, $P>0.10$, Table 8:1, page 161). There was a significant difference in the number of eggs *in utero* ($0.01<P<0.05$) for the two treatments in experiment 7, but this result was not duplicated in experiment 6. In neither of these experiments was there a significant difference in worm burden related to the two levels of priming.

There was a possible posterior migration of parasites in challenged rats which received cells primed against the larger infection dose in Expts 4 and 5, and in both groups in Expts 6 and 7 (Tables 7:2(iv)-(vii), page 336).

### 8.4. Homologous and heterologous challenge of rats given immune mesenteric lymph node cells

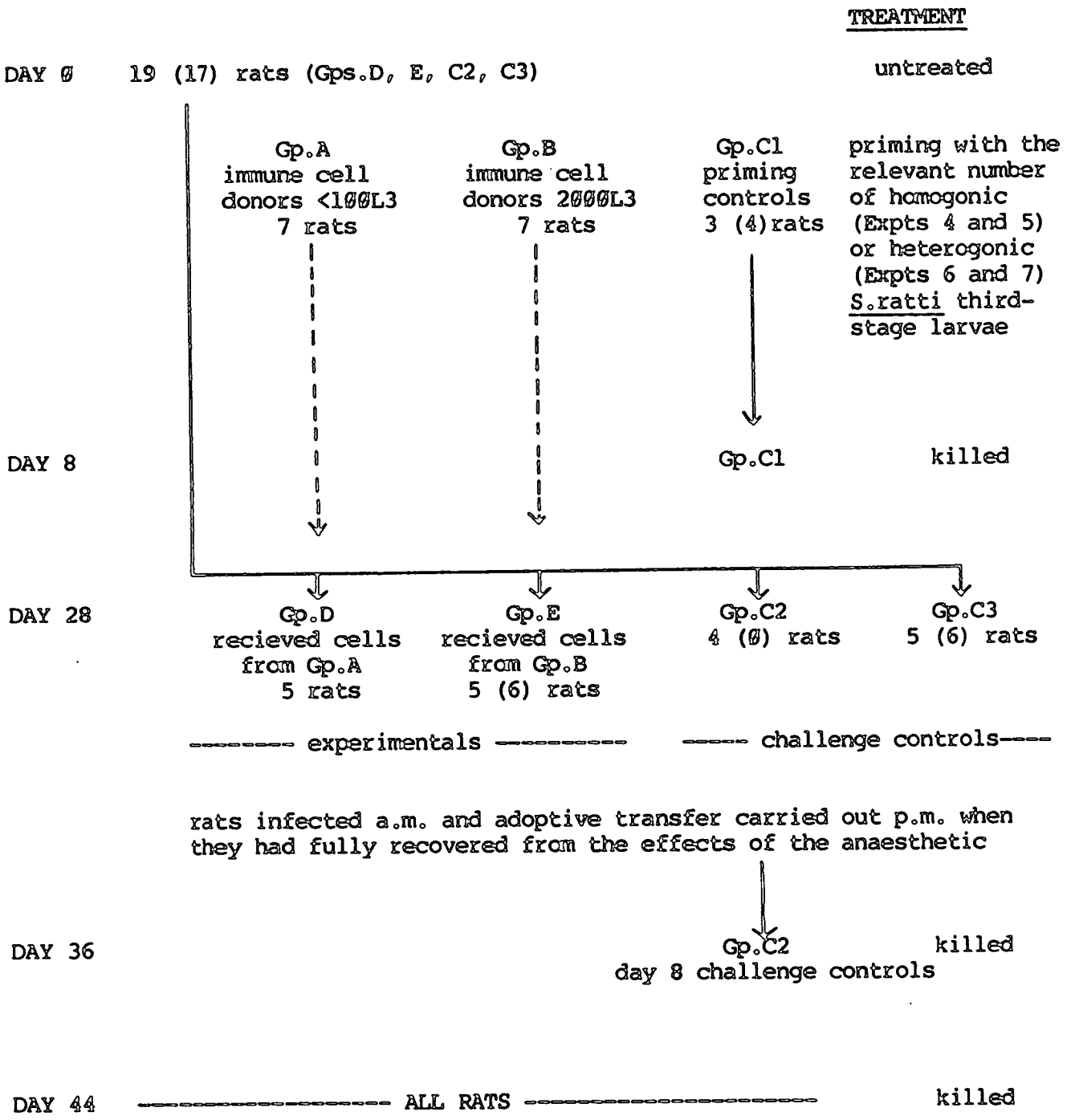
Since a significant immunity had been obtained using the protocol in Section 2, this procedure was used to carry out cross-resistance studies. Rats which supplied immune cells were infected with an estimated dose of 2000 third-stage *Strongyloides spp.* larvae since this dose gave a higher level of resistance in the recipients challenged with the homogonic strain of *S.ratti*

Fig.8:5

On day 0 rats in Gps.A, B and C1 were infected by skin-application with infective *S.ratti* larvae of the homogonic or heterogonic strain. An exact dose of <100 larvae was administered to rats in Gps.A and C1, whilst those in Gp.B were infected with an estimated dose of 2000 larvae. Eight days later the primary controls (Gp.C1) were killed to give an indication of the efficacy of priming. On day 28 post-primary infection both cell transfer and challenge infection was carried out and all rats were given an exact dose of <100 infective larvae of the homologous parasite, administered by skin-application. On day 8 post-challenge Gp.C2 rats were killed to check on the viability of larvae used at challenge and 8 days later all rats were killed. Group C2 was omitted from Expt 7 to economise on the number of rats used. Comparison of the proportion of the dose recovered and the number of eggs *in utero* per worm from animals in Gps. D and E showed whether altering the infection level in the cell donors affected the amount of immunity transferred. The number of rats shown were used in Expts 4, 5 and 6. The numbers shown in brackets refer to the numbers used in Expt 7.

Fig 8:5

Procedure used in Expts 4, 5, 6 and 7




KEY

——— indicates progress of the same animals

- - - - - - - indicates transfer of mesenteric lymph node cells

Table 8:1

Recipients of immune mesenteric lymph node cells (Gps.D and E) from cell donors; infected with either an exact dose of <100, or a mean of 2000, *S.ratti* infective larvae, were challenged with an exact dose of <100 third-stage larvae of the homologus parasite. Sixteen days later the rats were killed and the amount of protection induced found by comparing the worm burden and the egg data of the challenged groups (Gps.D and E) and the secondary controls (Gp.C3). The viability of the larvae used at both infections was assessed from the egg and worm data of day 8 controls (Gp.C1, priming infection; Gp.C2, challenge infection). In experiments 4 and 5 (Table 8:1a) the homogonic strain of *S.ratti* was the parasite used to infect animals whereas in Expts.6 and 7 (Table 8:1b) it was the heterogonic strain.

Table 8:1

a) Priming cell donors against the homogonic strain of S.ratti: homologous challenge of cell recipients assayed on day 16 post-infection

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 4 | homologous challenge | Gp.E | 0.027 | 3 | 0.021 | 1.750 | 3 | 1.250 |
| | homologous challenge | Gp.D | 0.137 | 5 | 0.058 | 3.929 | 5 | 0.535 |
| | priming controls | Gp.C1 | 0.655 | 3 | 0.088 | 7.283 | 3 | 0.029 |
| | day 8 challenge controls | Gp.C2 | 0.567 | 4 | 0.075 | 7.030 | 4 | 0.202 |
| | day 16 challenge controls | Gp.C3 | 0.292 | 5 | 0.065 | 6.441 | 5 | 0.516 |

mean exact dose — priming 95.20, challenge 97.12

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | 0.10>P>0.05 | P>0.25 |
| Gp.C2 vs C3 | P<0.001 | 0.001<P<0.01 |
| Gp.C3 vs D/E | P<0.001 | P<0.001 |
| Gp.D vs E | P<0.001 | P<0.001 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | homologous challenge | Gp.E | 0.121 | 6 | 0.069 | 2.810 | 6 | 1.337 |
| | homologous challenge | Gp.D | 0.304 | 5 | 0.064 | 5.825 | 5 | 0.402 |
| | priming controls | Gp.C1 | 0.562 | 3 | 0.146 | 7.933 | 4 | 0.605 |
| | day 8 challenge controls | Gp.C2 | 0.584 | 4 | 0.100 | 7.220 | 4 | 0.177 |
| | day 16 challenge controls | Gp.C3 | 0.441 | 4 | 0.079 | 7.288 | 4 | 0.210 |

mean exact dose — priming 93.80, challenge 96.79

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.25 | 0.10>P>0.05 |
| Gp.C2 vs C3 | 0.01<P<0.05 | P>0.25 |
| Gp.C3 vs D | P<0.001 | 0.001<P<0.01 |
| Gp.C3 vs E | P<0.001 | P<0.001 |
| Gp.D vs E | P<0.001 | P<0.001 |

Table 8:1

b) Priming cell donors against the heterogonic strain of S.ratti: homologous challenge of cell recipients assayed on day 16

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 6 | homologous challenge | Gp.E | 0.132 | 6 | 0.048 | 2.422 | 6 | 0.711 |
| | homologous challenge | Gp.D | 0.172 | 4 | 0.072 | 2.561 | 4 | 1.051 |
| | priming controls | Gp.C1 | 0.483 | 3 | 0.057 | 6.201 | 3 | 0.267 |
| | day 8 challenge controls | Gp.C2 | 0.517 | 4 | 0.054 | 6.304 | 4 | 0.469 |
| | day 16 challenge controls | Gp.C3 | 0.340 | 5 | 0.089 | 4.980 | 5 | 0.594 |

mean exact dose — priming 97.40, challenge 98.42

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.25 | P>0.25 |
| Gp.C2 vs C3 | P<0.001 | 0.001<P<0.01 |
| Gp.C3 vs D/E | P<0.001 | P<0.001 |
| Gp.D vs E | 0.25>P>0.10 | P>0.25 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | homologous challenge | Gp.E | 0.090 | 7 | 0.053 | 3.367 | 7 | 1.422 |
| | homologous challenge | Gp.D | 0.107 | 4 | 0.087 | 4.500 | 4 | 1.240 |
| | priming controls | Gp.C1 | 0.446 | 4 | 0.116 | 6.050 | 4 | 0.670 |
| | day 16 challenge controls | Gp.C3 | 0.277 | 4 | 0.095 | 7.075 | 4 | 0.401 |

mean exact dose — priming 97.64, challenge 93.88

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C3 | 0.001<P<0.01 | 0.10>P>0.05 |
| Gp.C3 vs D/E | P<0.001 | P<0.001 |
| Gp.D vs E | P>0.25 | 0.01<P<0.05 |

This may increase the sensivity of the assay and aid in detecting differences in the host's immune response against different types of parasite.

### 8.4.1. Materials and methods

The general protocol shown in Fig.8:6 (page 164) was followed in experiments 8-13.

#### 8.4.1.1. Expts 8 and 9

The cells used at transfer ($2.5x10^8$ cells per rat) were primed against the homogonic strain of *S.ratti* and recipients were infected either with the homologous or heterologous *S.ratti* strain.

#### 8.4.1.2. Expts 10 and 11

The reciprocal experiment to that carried out in Expts 8 and 9 was undertaken, thus cells were primed against the heterogonic strain of *S.ratti*. The number of cells each animal received in Expts 10 and 11 were $2.5x10^8$ and $2.0x10^8$ respectively. The smaller inoculum had to suffice since the yield from the donors in that case was insuffient to provide the standard dose.

#### 8.4.1.3. Expts 12 and 13

The cells used at transfer were primed against *S.venezuelensis* and rats which received the cells were challenged with the homologous parasite or one of the two strains of *S.ratti*. In Expt 12 each rat received $1.55x10^8$ cells whereas in Expt 13 $2.5x10^8$ cells were administered.

After killing rats at the appropriate time, the number of adults present,

their distribution within the intestine, and the number of eggs *in utero* per worm for each rat was found.

### 8.4.2. Results

A significant resistance to both homologous and heterologous challenge was induced in all experiments regardless of the strain or species used to prime rats. This was demonstrated in rats given immune mesenteric lymph node cells as a significant reduction in the proportion of the dose recovered, and, with one exception, a significant reduction in the number of eggs *in utero* per worm, compared with corresponding controls (Table 8:2, pages 166 and 167). The exception was Expt 12, where there was no difference in the mean number of eggs *in utero* for each worm from secondary homogonic *S.ratti* controls and the corresponding challenge treatment (Gp.D vs Gp.C3, $\underline{P}>0.25$). In the duplicate experiment a significant difference between the two treatments was obtained ($0.001<\underline{P}<0.01$).

In addition, there seemed to be a quantitative difference in the level of immunity obtained after challenge with *S.ratti*, which depended on the strain used to prime rats.

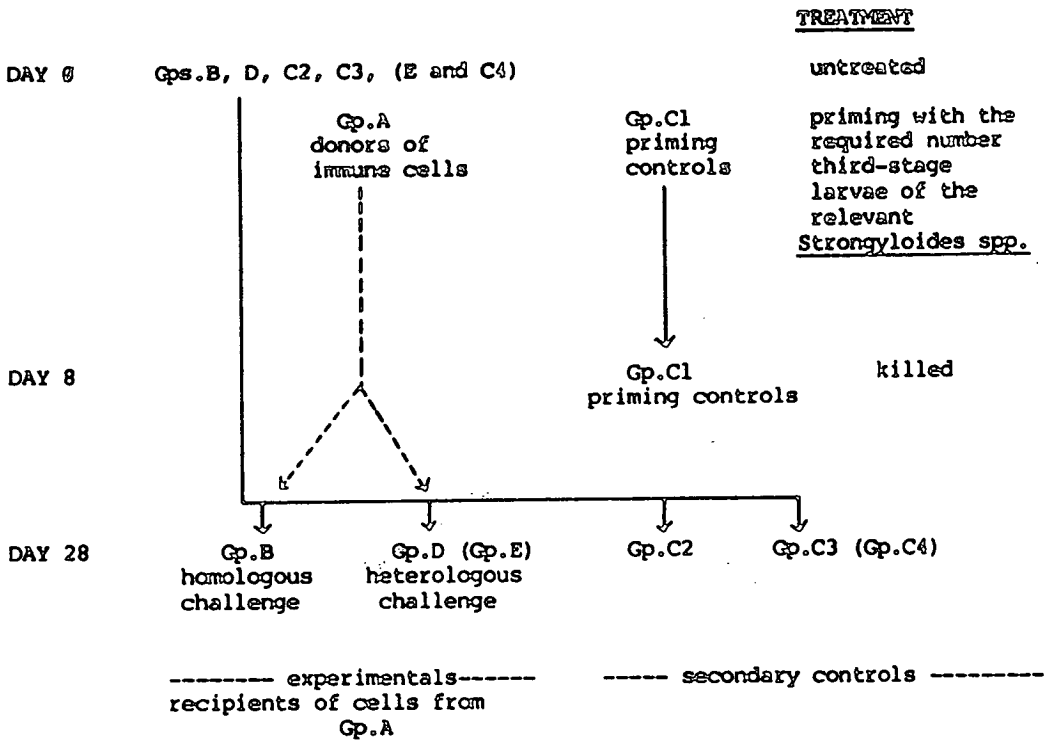
If the homogonic strain was used to prime the transferred immune cells there was no significant difference in the level of immunity produced against homologous or heterologous challenge in the recipients (Fig.8:7a, page 168). However if the heterogonic strain was used to immunise cell donors there was significantly greater resistance produced after homologous compared with heterologous challenge in Expt 10. This was demonstrated as a greater reduction in the proportion of the dose recovered ($0.01<\underline{P}<0.05$, Fig.8:7b(i), page 169). A corresponding differential reduction in number of eggs *in utero*

Fig.8:6

Rats in groups A (cell donors) and C1 (priming controls) were infected by skin-application with the appropriate strain of *S.ratti* or *S.venezuelensis*. An estimated dose of 2000 infective larvae were administered to Gp.A animals, whereas those in Gp.C1 were infected with an exact dose of $<100$ larvae. On day 8 post-infection the priming controls were killed to assess the viability of the larvae used. Challenge infection with an exact dose of $<100$ third-stage larvae of the relevant parasite and adoptive transfer of the immune mesenteric lymph node cells (prepared from rats in Gp.A) was carried out on day 28 post-infection. Animals in Gps. B and C1 were infected with the homologous strain or species used to prime cell donors whilst those in Gps. D and C2 were both infected with the same heterologous parasite. In Expts 12 and 13 there were two heterologous challenge treatments therefore an additional experimental group (Gp.E) and its corresponding control (Gp.C4) were included in the protocol.

Fig.8:6

Protocol used in Expts 8, 9, 10, 11, 12 and 13




Number of rats used

| Group | EXPT | | | | | |
|---|---|---|---|---|---|---|
| | 8 | 9 | 10 | 11 | 12 | 13 |
| Gp.A ▫ | 16 | 14 | 16 | 16 | 21 | 21 |
| Gp.C1 ▫ | 4 | 4 | 4 | 3 | 4 | 3 |
| Gp.C2 ▫ | 5 | 5 | 5 | 5 | 5 | 5 |
| Gp.C3 ▫ | 5 | 5 | 5 | 5 | 5 | 5 |
| Gp.C4 ▫ | | | | | 5 | 5 |
| Gp.B ▫ | 6 | 7 | 6 | 6 | 5 | 5 |
| Gp.D ▫ | 6 | 7 | 6 | 6 | 5 | 5 |
| Gp.E ▫ | | | | | 5 | 5 |

KEY

_____ indicates progress of the same animals

------- indicates transfer of mesenteric lymph node cells

per worm was not obtained. In the duplicate experiment (Expt 11) this situation was reversed, there was no significant difference in the proportion of the dose recovered from the two treatments but there was a significantly greater reduction in egg data after homologous challenge ($0.01<\underline{P}<0.02$, Fig.8:7b(ii), page 169).

If *S.venezuelensis* was used to prime cell donors, and worm burdens were taken as the criterion, there was no detectable difference in the level of immunity produced in the animals which received the cells after homologous or heterologous challenge. However, results for the egg data, which seem to be a more sensitive test of an anti-worm response, lead to the conclusion that the host's immune response could differentiate between the three parasites. Thus the greatest reduction in the mean number of eggs *in utero* occurred after homologous challenge. The next most effective treatment was heterologous challenge with heterogonic *S.ratti*, and the least one was challenge with homogonic *S.ratti* (Fig.8:8, page 171). There was an exception in Expt 12, where there was no significant difference in the relative reduction in the number of eggs *in utero* for the homologous and heterologous (heterogonic) *S.ratti* treatments, but the resistance quotient for the homologous treatment was numerically higher.

There seemed to be a possible posterior migration of adult *S.ratti* in both challenged groups from experiments 8 and 9, whereas it only seemed to occur after homologous challenge in experiments 10 and 11 (Tables 7:2(viii)-(xi), Appendix 7, page 338). There did not seem to be any adjustment in the relative location of adults from any treatment in experiments 12 and 13 (Tables 7:2(xii) and (xiii), Appendix 7, page 341) where *S.venezuelensis* was used to prime the cells used at transfer.

Table 8:2

Rats which had received immune mesenteric lymph node cells from donors, infected with a mean dose of 2000 third-stage *Strogyloides spp.* larvae, were challenged with an exact dose of <100 infective larvae of the homologous or heterologous parasite. Sixteen days later the rats were killed and the amount of protection induced assessed by comparing the worm and egg data from the challenged rats (Gps.B, D and E) with their corresponding controls (Gps.C2, C3 and C4 respectively). The parasites used in each experiment were: Expts 8 and 9, homogonic strain of *S.ratti* priming cell donors, homogonic or heterogonic strain of *S.ratti* challenging recipients (Table 8:2a); Expts 10 and 11, heterogonic strain of *S.ratti* priming cell donors, heterogonic or homogonic strain of *S.ratti* challenging recipients (Table 8:2b); Expts 12 and 13, *S.venezuelensis* priming cell donors, *S.venezuelensis* or *S.ratti* (homogonic and heterogonic strains) challenging recipients (Table 8:2c).

Table 8:2

a) Donors of cells primed against the homogonic strain of S.ratti: effect of homologous and heterologous challenge with the heterogonic strain on the cell recipients

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 8 | homologous challenge | Gp.B | 0.165 | 6 | 0.106 | 3.295 | 6 | 2.067 |
| | heterologous challenge | Gp.D | 0.156 | 6 | 0.066 | 2.425 | 6 | 0.674 |
| | priming controls | Gp.C1 | 0.647 | 4 | 0.074 | 7.554 | 4 | 0.291 |
| | challenge controls homogonic S.ratti | Gp.C2 | 0.535 | 5 | 0.065 | 6.748 | 5 | 0.232 |
| | challenge controls heterogonic S.ratti | Gp.C3 | 0.311 | 5 | 0.096 | 4.969 | 5 | 0.643 |
| | mean exact dose | priming | homogonic S.ratti | 95.25 | | | | |
| | | challenge | homogonic S.ratti | 95.64 | | | | |
| | | | heterogonic S.ratti | 94.82 | | | | |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | 0.01<P<0.05 | 0.25<P<0.10 |
| Gp.C2 vs C3 | P<0.001 | 0.001<P<0.01 |
| Gp.C2 vs B | P<0.001 | P<0.001 |
| Gp.C3 vs D | P<0.001 | P<0.001 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9 | homologous challenge | Gp.B | 0.152 | 7 | 0.059 | 4.055 | 7 | 1.093 |
| | heterologous challenge | Gp.D | 0.131 | 7 | 0.075 | 3.697 | 7 | 0.980 |
| | priming controls | Gp.C1 | 0.742 | 4 | 0.110 | 7.411 | 4 | 0.379 |
| | challenge controls homogonic S.ratti | Gp.C2 | 0.379 | 4 | 0.150 | 6.538 | 4 | 0.975 |
| | challenge controls heterogonic S.ratti | Gp.C3 | 0.268 | 5 | 0.094 | 6.128 | 5 | 0.461 |
| | mean exact dose | priming | homogonic S.ratti | 95.50 | | | | |
| | | challenge | homogonic S.ratti | 96.09 | | | | |
| | | | heterogonic S.ratti | 96.75 | | | | |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P<0.001 | 0.01<P<0.05 |
| Gp.C2 vs C3 | 0.10>P>0.05 | P>0.25 |
| C2 vs B | P<0.001 | P<0.001 |
| C3 vs D | 0.001>P<0.01 | P<0.001 |

Table 8:2

b) Priming of cell donors with the heterogonic strain: effect of homologous and heterologous challenge with homogonic S.ratti on the cell recipients

| Expt | Treatment | Group | Mean Prop. of the dose recovered | n | SD | Mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 10 | homologous challenge | Gp.B | 0.077 | 4 | 0.050 | 3.075 | 4 | 1.951 |
| | heterologous challenge | Gp.D | 0.256 | 6 | 0.077 | 4.538 | 6 | 0.035 |
| | priming controls | Gp.C1 | 0.617 | 3 | 0.084 | 6.452 | 3 | 0.253 |
| | challenge controls heterogonic S.ratti | Gp.C2 | 0.338 | 5 | 0.044 | 5.279 | 5 | 0.345 |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.564 | 5 | 0.024 | 6.465 | 5 | 0.150 |
| | mean exact dose | priming | heterogonic S.ratti | 93.50 | | | | |
| | | challenge | heterogonic S.ratti | 97.64 | | | | |
| | | | homogonic S.ratti | 94.55 | | | | |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P<0.001 | 0.01<P<0.05 |
| Gp.C2 vs C3 | P<0.001 | 0.01<P<0.05 |
| Gp.C2 vs B | P<0.001 | P<0.001 |
| Gp.C3 vs D | P<0.001 | P<0.001 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11 | homologous challenge | Gp.B | 0.041 | 5 | 0.024 | 2.627 | 5 | 0.059 |
| | heterologous challenge | Gp.D | 0.037 | 5 | 0.051 | 4.019 | 4 | 1.269 |
| | priming controls | Gp.C1 | 0.493 | 3 | 0.072 | 6.362 | 3 | 0.470 |
| | challenge controls heterogonic S.ratti | Gp.C2 | 0.252 | 4 | 0.021 | 6.137 | 4 | 0.521 |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.297 | 4 | 0.063 | 6.900 | 4 | 0.745 |
| | mean exact dose | priming | heterogonic S.ratti | 95.33 | | | | |
| | | challenge | heterogonic S.ratti | 96.67 | | | | |
| | | | homogonic S.ratti | 96.67 | | | | |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P<0.001 | P>0.25 |
| Gp.C2 vs C3 | P>0.25 | 0.10>P>0.05 |
| Gp.C2 vs B | P<0.001 | P<0.001 |
| Gp.C3 vs D | P<0.001 | P<0.001 |

Table 8:2

c) S.venezuelensis priming cell donors: effect of homologous and heterologous challenge with S.ratti on cell recipients

| Expt | Treatment | Group | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|
| 12 | homologous challenge | Gp.B | 0.111 | 5 | 0.057 | 4.386 | 4 | 1.101 |
| | heterologous challenge homogonic S.ratti | Gp.D | 0.307 | 5 | 0.124 | 6.855 | 5 | 0.785 |
| | heterologous challenge heterogonic S.ratti | Gp.E | 0.183 | 5 | 0.054 | 4.323 | 5 | 0.831 |
| | priming controls | Gp.C1 | 0.269 | 4 | 0.159 | 6.811 | 3 | 0.275 |
| | challenge controls S.venezuelensis | Gp.C2 | 0.272 | 5 | 0.088 | 6.211 | 5 | 0.732 |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.399 | 5 | 0.079 | 7.010 | 5 | 0.412 |
| | challenge controls heterogonic S.ratti | Gp.C4 | 0.352 | 5 | 0.064 | 5.266 | 5 | 0.643 |
| | mean exact dose | priming | S.venezuelensis | | 94.75 | | | |
| | | challenge | S.venezuelensis | | 97.11 | | | |
| | | | homogonic S.ratti | | 95.20 | | | |
| | | | heterogonic S.ratti | | 95.11 | | | |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.25 | 0.10>P>0.05 |
| Gp.C2 vs C3 | 0.01<P<0.05 | 0.01<P<0.05 |
| Gp.C2 vs C4 | 0.10>P>0.05 | 0.001<P<0.01 |
| Gp.C3 vs C4 | P>0.25 | P<0.001 |
| Gp.C2 vs B | P<0.001 | P<0.001 |
| Gp.C3 vs D | 0.01<P<0.05 | P>0.25 |
| Gp.C4 vs E | P<0.001 | 0.001<P<0.01 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13 | homologous challenge | Gp.B | 0.143 | 4 | 0.052 | 3.969 | 4 | 1.117 |
| | heterologous challenge homogonic S.ratti | Gp.D | 0.336 | 5 | 0.111 | 6.747 | 5 | 0.461 |
| | heterologous challenge heterogonic S.ratti | Gp.E | 0.181 | 4 | 0.044 | 4.917 | 4 | 0.289 |
| | priming controls | Gp.C1 | 0.368 | 3 | 0.093 | 6.031 | 3 | 0.219 |
| | challenge controls S.venezuelensis | Gp.C2 | 0.320 | 5 | 0.148 | 6.788 | 5 | 0.331 |
| | challenge controls homogonic S.ratti | Gp.C3 | 0.473 | 3 | 0.010 | 7.483 | 3 | 0.208 |
| | challenge controls heterogonic S.ratti | Gp.C4 | 0.316 | 5 | 0.078 | 6.015 | 5 | 0.273 |
| | mean exact dose | priming | S.venezuelensis | | 88.33 | | | |
| | | challenge | S.venezuelensis | | 96.78 | | | |
| | | | homogonic S.ratti | | 97.67 | | | |
| | | | heterogonic S.ratti | | 93.78 | | | |

| P values | worm data | egg data |
|---|---|---|
| Gp.C1 vs C2 | P>0.25 | P>0.25 |
| Gp.C2 vs C3 | 0.01<P<0.05 | 0.001<P<0.01 |
| Gp.C2 vs C4 | P>0.25 | P<0.001 |
| Gp.C3 vs C4 | 0.01<P<0.05 | P<0.001 |
| Gp.C2 vs B | 0.001<P<0.01 | P<0.001 |
| Gp.C3 vs D | 0.01<P<0.05 | 0.001<P<0.01 |
| Gp.C4 vs E | 0.001<P<0.01 | P<0.001 |

Fig.8:7

The amount of resistance produced in rats after homologous and heterologous challenge was compared by calculating resistance quotients for the two treatments. In Expts 8 and 9 the homogonic strain of *S.ratti* was used to prime the cell donors, whereas in Expts 10 and 11 the donors were primed against the heterogonic strain.

Fig.8:7

Comparison of the amount of protection induced in rats after homologous ar

heterologous challenge: S.ratti priming cell donors, Expts 8-11

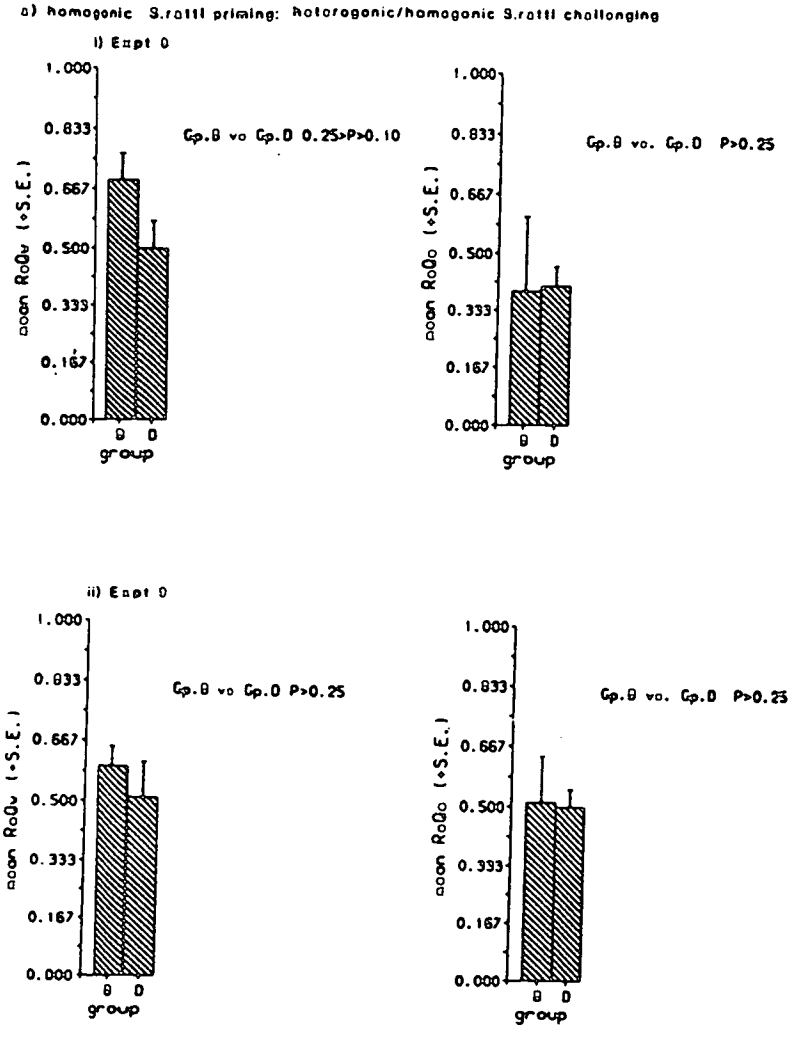
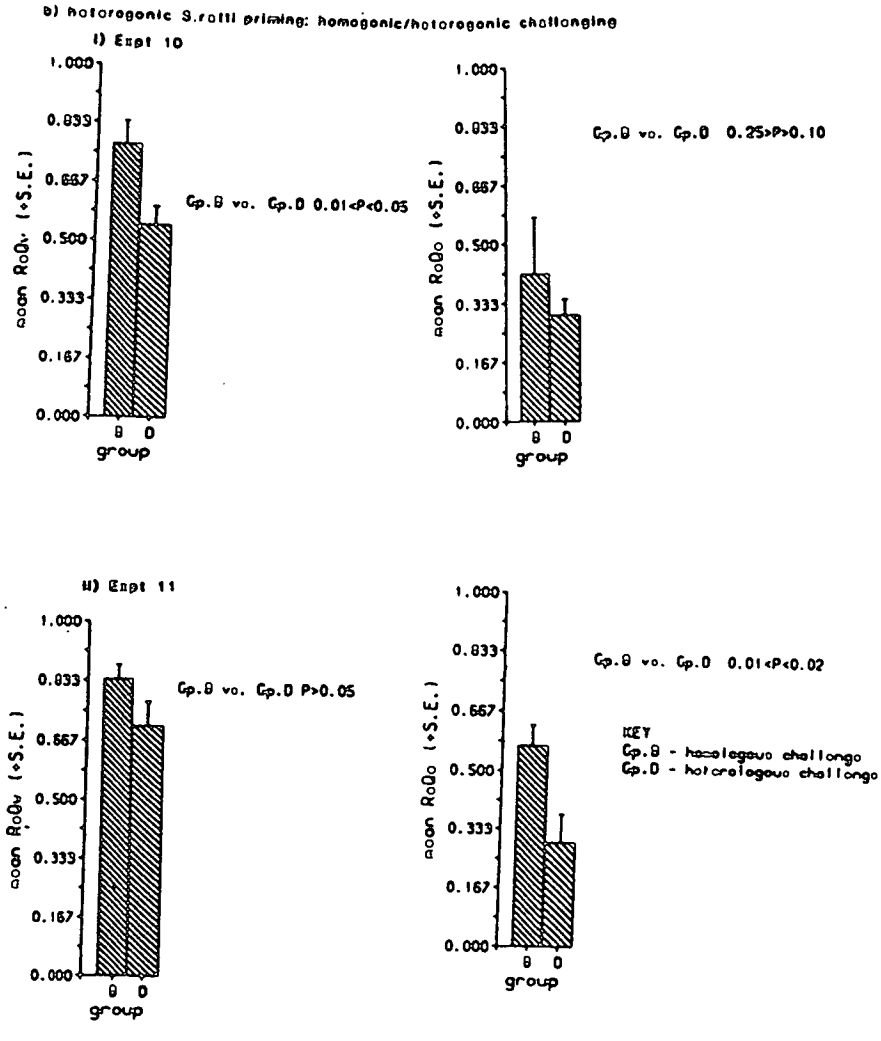


Fig.8:7 (continued)



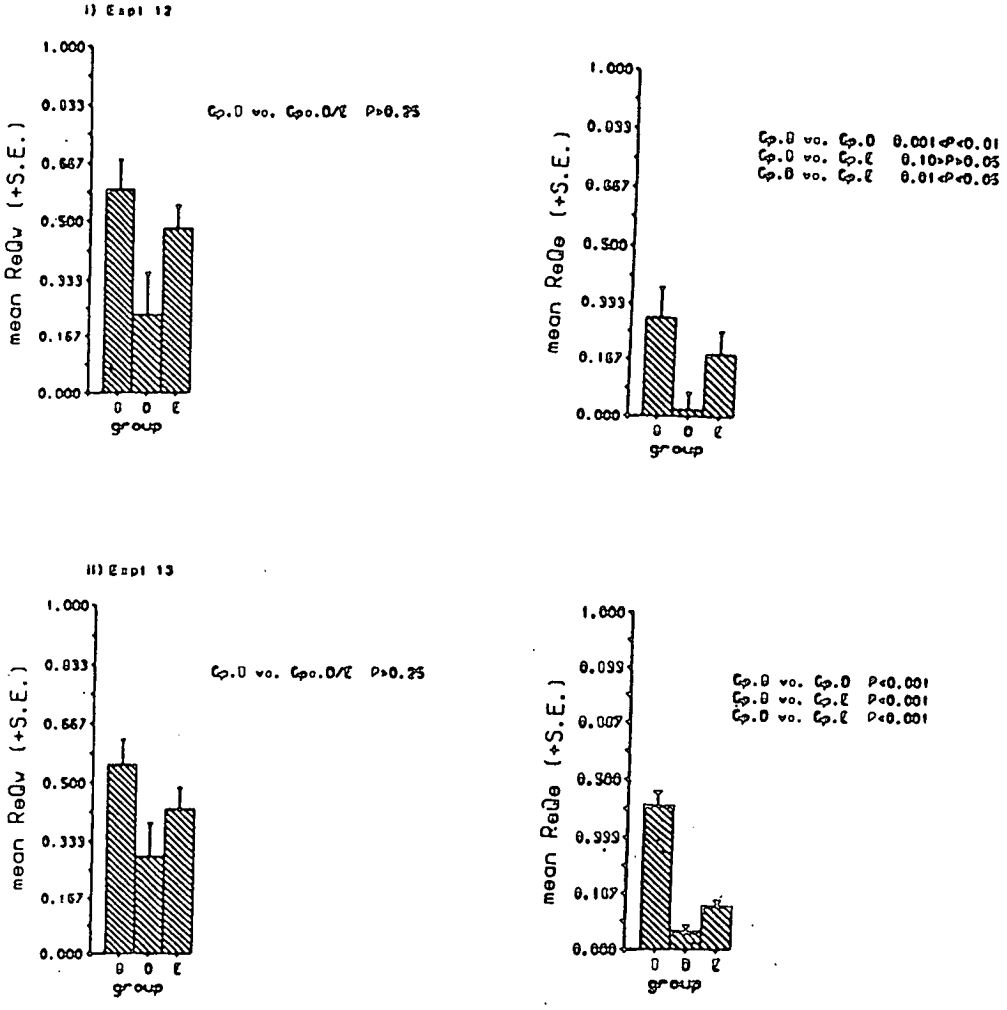
Details of the methods used are given in the relevant section of Chapter 2 (section 2:10, for the method of infection and worm counting; section 2:5, for the method used at adoptive transfer; and section 2:11, for statistical analysis of the results, pages 47, 37, and 51 respectively).

Fig.8:8

The amount of protection induced in rats after homologous and heterologous challenge was assessed by calculating resistance quotients for the egg and worm data. The animals had received immune mesenteric lymph node cells, from donors infected with *S.venezuelensis*, and were challenged with an exact dose of <100 infective larvae of the relevant parasite.

Fig.8:8

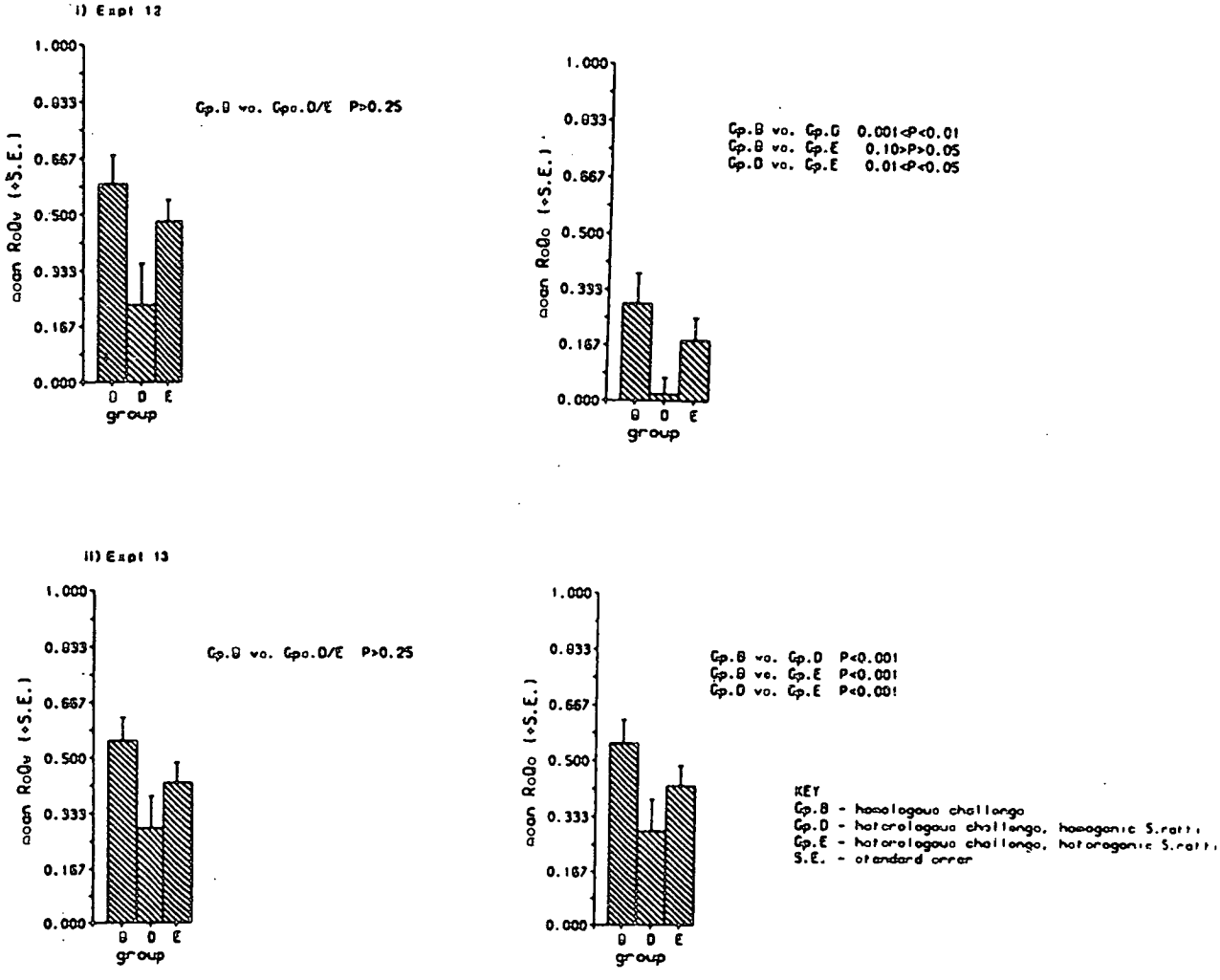
Comparison of the amount of resistance induced in animals after homologous and heterologus challenge: S.venezuelensis priming cell donors, Expts 12 and 13




KEY
Gp.B - homologous challenge
Gp.D - heterologous challenge, homogonic S.ratti
Gp.E - heterologous challenge, heterogonic S.ratti
S.E. - standard error

Fig.8:8

Comparison of the amount of resistance induced in animals after homologous and heterologus challenge: S.venezuelensis priming cell donors, Expts 12 and 13



## 8.5. Summary

a) The immunity adoptively transferred was not firmly expressed until day 16 post-infection. If rats were killed on day 8, there was no consistent demonstrable resistance to re-infection, even if the infection dose used to infect rats which provided the cells, was increased.

b) Immune mesenteric cells primed against an exact dose of less than 100 third-stage *S.ratti* larvae are capable of immunising rats against homologous challenge.

c) A dose-response effect was demonstrated if rats were immunised with transferred mesenteric lymph node cells from donors infected with different dose levels of *S.ratti* homogonic strain third-stage larvae (i.e. an exact dose of $<100$ larvae vs 2000 larvae), which was expressed as a greater reduction in the proportion of the dose recovered, and a greater reduction in the number of eggs *in utero* per worm, after homologous challenge. A corresponding effect was not obtained if the heterogonic strain was used to prime cells.

d) There seemed to be an asymmetry in the level of immunity expressed against homologous and heterologous challenge in rats immunised with immune mesenteric lymph node cells, which depended on the strain of *S.ratti* used to prime the cells. If the heterogonic strain was used homologous challenge produced the greater resistance compared with heterologous challenge with the homogonic strain; demonstrated as a significantly greater reduction in the proportion of the dose recovered in Expt 10, and a significantly greater reduction in the egg data in Expt 11. In contrast, if the homogonic strain was used to prime the cells there was no significant difference in these two parameters after homologous and heterologous

challenge.

e) Adoptive transfer of mesenteric lymph node cells primed against *S.venezuelensis* protected rats against homologous and heterologous *S.ratti* infection. If the egg data was considered there seemed to be a quantitative difference in the level of immunity induced at challenge. Maximum protection was elicited after homologous challenge, followed by heterologous challenge with the heterogonic strain of *S.ratti*, and the minimum level of immunity was expressed after heterologous challenge with the homogonic strain (assessed as the relative reduction in the number of eggs *in utero* per worm for the three groups). If the worm data was considered there was no difference in the resistance produced after challenge with any parasite.

f) There did not seem to be any clear difference in the longitudinal distribution of *Strongyloides spp.* parasites from challenged and control treatments.

# CHAPTER 9

# THE AMOUNT OF LYMPHOCYTE STIMULATION PRODUCED BY *STRONGYLOIDES SPP.* ANTIGENS

## 9.1. Introduction

Lymphocyte stimulation or transformation can be induced either by the interaction of lymphocytes with specific antigen, or with certain plant products, called lectins, causing the production of blast cells. The mode of activation in both cases is thought to be similar, although stimulation by an antigen leads to the proliferation of specific clones of lymphocytes, whereas lectin induced mitogenesis is non-specific and thus polyclonal. The amount of proliferation induced is assumed to be related to DNA synthesis, judged in practice by the level of incorporation of a radio-labelled nucleotide, such as tritiated thymidine. Thus comparative studies of the relative stimulation induced by various antigenic preparations seemed possible.

Initially the assay was used to investigate four aspects of the cellular response to *Strongyloides spp.* as follows:-

a) the amount of cross-reactivity between the three *Strongyloides spp.* parasites by priming the donors of cells against antigens from one of them followed by homologous and heterologous challenge.

b) the stage-specificity of the response by challenging *in vitro* cells from primed donors using antigenic preparations made from different stages of the life-cycle.

c) the nature of the antigens involved in eliciting an immune response by

comparing the response to somatic and excretory/secretory antigen preparations.

d) the dynamics of the cellular response by challenging cells taken at different times post-infection with the same antigen preparation. Unfortunately the antigens prepared could not be standardised since they were found to contain no detectable protein as judged by the Lowry assays carried out (see Appendix 8:5, page 357). Rather than abandon these studies altogether the preparations were tested to find if they did have antigenic properties. It was possible that they might have some specific mitogenic effect on primed cells since some protein may have been present but at too low a concentration for the Lowry assay to detect it, or that other substances such as carbohydrates, whose concentration had not assessed, may elicit a response in primed cells. Different dilutions of the antigens were assayed in some experiments to find if there was a dose-response effect, which would confirm that antigens were present. A similar difficulty in removing protein from *S.ratti* has been described by Murrell and Graham (1982) who tried to strip epicuticular antigens from the surface of third-stage *S.ratti* larvae. Treatment with a variety of enzymes, including proteases, carbohydrases and lipase, as well as detergents, failed to remove any material from the surface of the larvae.

Previous results by Genta *et al.* (1983) have shown that, among those tested, the most responsive cell population to a *S.ratti* antigen was the mesenteric lymph node cells. Therefore this cell population was used throughout the experiments. As a positive control, treatment with the T cell mitogen, concanavalin A, was also included in the experimental protocol to show that cells were capable of responding to stimulation. The response of

unstimulated cells was also recorded to give an indication of the amount of proliferation initiated *in vivo*.

Each experiment carried out in this section is described separately since different combinations of antigens were tested. However, the same general technique described in Section 2:6, Chapter 2, page 38 was used throughout. Other methods common to these experiments used are given on page 213.

## 9.2. Dynamics of the response

The timing of the peak cellular response against *Strongyloides spp.* parasites was determined by investigating the response of mesenteric lymph node cells, taken at various times during a primary infection, to antigenic stimulation.

### 9.2.1. Expt 1, Dynamics of the cellular response: heterogonic strain of *S.ratti* priming cell donors

#### 9.2.1.1. Materials and methods

Eighteen rats were divided into 3 groups:-

Gp.X - 9 animals - infected by skin-application with 2000 third-stage heterogonic strain *S.ratti* larvae, to provide immune mesenteric lymph node cells.

Gp.Y - 6 rats - uninfected, provided normal mesenteric lymph node cells.

Gp.Z - 3 rats - controls to assess the viability of the larvae, infected in the same manner as Gp A using an exact dose of $<100$ larvae and killed on day 8

post-infection. Three rats from Gp.X and two rats from Gp.Y were killed on days 8, 16 and 28 post-infection. The mesenteric lymph node cells obtained from them were plated out as shown in Fig.9:1, page 179. Numbers of worms and their distribution along the gut were also recorded.

9.2.1.2. Results

A significantly higher level of unstimulated activity was obtained in immune cells ($0.01<\underline{P}<0.05$) obtained on day 8 post-infection (Fig.9:2a, page 180). Unstimulated activity of cells from day 16 of a primary infection illustrated a feature which recurred throughout these assays: that is to say, cells from different rats within treatments were often significantly different in their behaviour. Thus, in this case, although the overall difference between cells from uninfected rats and those from infected ones at day 16 was not significant (Fig.9:2a, page 180), 2 of the 3 infected rats did have a significantly higher activity than any of the controls ($P<0.001$). This within-treatment variation often presented a problem in interpreting the results (see Discussion). On day 28 post-infection there was obviously no difference in the unstimulated blastogenesis of the two cell populations (Fig.9:2a, page 180) and no idiosyncracy within treatments.

Cells from infected and uninfected rats on day 8 and 16 post-infection expressed a similar level of mitogen-induced activation (Fig.9:2b, page 180). In contrast day 28 cells from immune rats expressed a significantly lower response to concanavalin A stimulation ($0.01<\underline{P}<0.025$), Fig.9:2b, page 180).

There was no specific stimulation of day 8 immune cells but day 16 cells from one of the three infected rats gave a positive response to homologous challenge (Fig.9:2c, page 181). On day 28 post-infection there was no

response to the neat heterogonic strain *S.ratti* antigen, therefore a comparison of the response of immune cells to the different concentrations of the antigen is omitted.

The worm data (Table 8:2(i), Appendix 8, page 352) showed there was no significant difference in the proportion of the dose recovered from rats infected either with an exact dose of <100, or 2000, third-stage heterogonic strain *S.ratti* larvae on day 8 post-infection. Over the course of infection the worm burden in the cell donors fell, until day 28 post-infection when no parasites were recovered. A corresponding reduction in the number of eggs *in utero* per worm was obtained on day 16 post-infection, and there also seemed to be a posterior migration of adults compared with the results for day 8 post-infection (Table 8:3(i), Appendix 8, page 354).

### 9.2.2. Expt 2, The response of day 28 immune cells to various treatments: heterogonic strain of *S.ratti* priming cell donors

#### 9.2.2.1. Materials and methods

Five rats were divided in to two groups:-

Gp.X - 3 rats - infected by skin-application with 2000 infective *S.ratti* larvae of the heterogonic strain and killed 28 days later to provide immune cells.

Gp.Y - 2 rats - untreated animals which provided normal mesenteric lymph node cells.

On day 28 of Gp.X's infection the rats were killed and the cells obtained plated out as shown in Fig.9:3, page 183. The number of adult worms present

Fig.9:1

On days 8 and 16 cells obtained from two of any of the five rats were plated out together as shown in (i). Therefore there was a possibility that a plate could contain cells from an uninfected (Gp.Y) and infected rat (Gp.X). Since there was an odd number of animals, a separate plate had to be set up for one of the rats. In this case, its cells were added to all wells but columns 5-12 which contained cells and medium were ignored. On day 28 a separate plate was set up for each individual rat (ii). Only results for cells exposed to treatments U, C, A1, A2 and A3 were recorded.

Fig.9:1

Appearance of microtitre plate in Expt 1

i) Days 8 and 16

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | C | A | m | U | C | A | m | m | m | m |
| | C | m | U | C | A | m | U | C | A | m | m | m | m |
| | D | m | U | C | A | m | U | C | A | m | m | m | m |
| | E | m | U | C | A | m | U | C | A | m | m | m | m |
| | F | m | U | C | A | m | U | C | A | m | m | m | m |
| | G | m | U | C | A | m | U | C | A | m | m | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

<---- cells from a rat ---><------ cells from another rat ------->

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium but the cells were harvested)
C = concanavalin A added to cells (2.5µg/ml)
A = cells treated with adult heterogonic strain of S.ratti excretory/secretory antigen

ii) Day 28

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | C | A1 | A2 | A3 | U | C | A1 | A2 | A3 | m |
| | C | m | U | C | A1 | A2 | A3 | U | C | A1 | A2 | A3 | m |
| | D | m | U | C | A1 | A2 | A3 | U | C | A1 | A2 | A3 | m |
| | E | m | U | C | A1 | A2 | A3 | U | C | A1 | A2 | A3 | m |
| | F | m | U | C | A1 | A2 | A3 | U | C | A1 | A2 | A3 | m |
| | G | m | U | C | A1 | A2 | A3 | U | C | A1 | A2 | A3 | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

<---- cells from a rat --------><---- cells from another rat ----->

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium but the cells were harvested)
C = concanavalin A added to cells (2.5µg/ml)
A = cells treated with adult heterogonic strain of S.ratti excretory/secretory antigen
1 = neat antigen
2 = antigen diluted 1:2
3 = antigen diluted 1:4

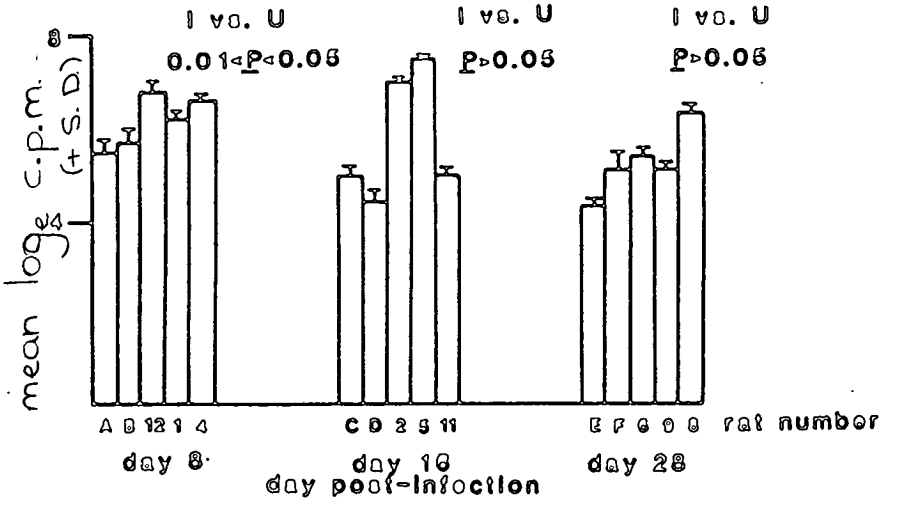
Fig.9:2

The effect of addition of medium alone (unstimulated cells, treatment U, Fig.9:2a), mitogen (treatment C, Fig.9:2b) and homologous excretory/secretory worm antigen (treatment A, Fig.9:2c) on the level of stimulation of cells from donors either untreated or primed with the heterogonic strain of *S.ratti* was investigated. Each bar in the figure refers to the results of cells obtained from an individual animal, rats A-F were uninfected (Gp.Y) and those numbered 1-12 were infected (Gp.X). The p values shown in the figures refer to the overall comparison of the response of cells obtained from uninfected and uninfected donors (Gp.X versus Gp.Y, shown as I vs U, where I stands for infected and U for uninfected) to the various treatments.

Fig.9:2

Cellular response to various treatments, heterogenic strain of S.ratti priming cell donors, Expt 1

a) Unstimulated cellular activity




b) Concanavalin A

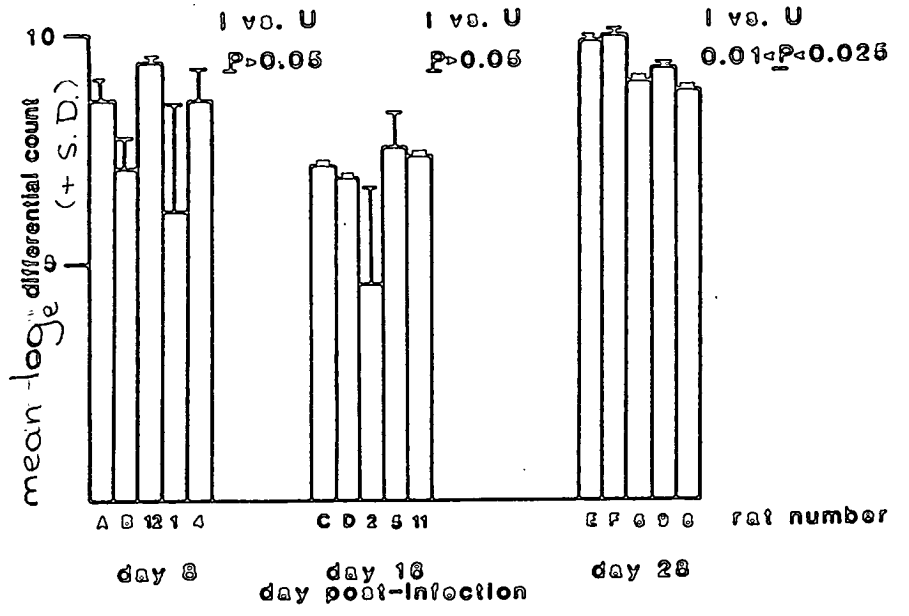
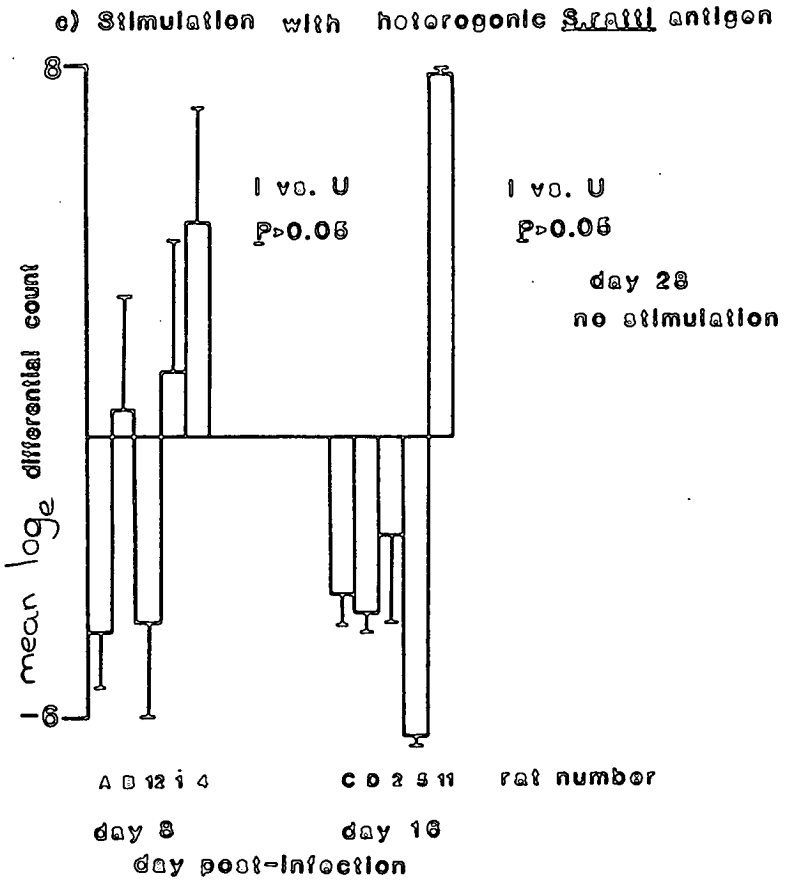


Fig. 9:2 (continued)



in the cell donors, their distribution along the intestine and the number of eggs *in utero* per worm was recorded. To economise on the number of rats, day 8 controls, which give an indication of the viability of the larvae used at priming were omitted.

9.2.2.2. Results

There was no significant difference in unstimulated activity of normal and immune cells (Fig.9:4a, page 185). The overall treatment difference in the amount of concanavalin A induced blastogenesis of cells from normal and immune rats was not significant statistically (using the data of cells treated with 2.5ug/ml of concanavalin A since this was used throughout other experiments) but there were highly significant differences in the results from individual animals ($P<0.01$, Fig.9:4b, page 185). If compared on a rat to rat basis, and applying the least significant differences between means, the low response of immune cells compared with non-immune was highly significant ($P<0.001$). The biggest mean among infected rats was less than half the value of the smallest from uninfected animals (Table 8:1(iv), Appendix 8, page 345 ).

In regard to the response of normal cells to different concentrations of concanavalin A, two-way analysis of variance identified significant interaction between rats and concanavalin A concentration ($P<0.05$, Fig.9:4b, page 185). Using one way analyses of variance of the data for each animal, rat A had a significantly higher response to 5µg/ml ($P<0.001$), but there was no difference in its response to 2.5 or 10 µg/ml. Rat B seemed to have a graded response to concanavalin A with a significant difference between the two extreme concentrations ($P<0.05$).

Cells from immune rats showed no overall significant difference in

Fig.9:3

Cells collected from each of the five rats were dispensed into a separate microtitre plate and then the relevant sample was added to each well as shown. Only results for treatments U, HO, HE, S, C1, C2 and C3 were recorded.

Fig.9:3

Appearance of microtitre plate in Expt 2

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | HO | HE | S | C1 | C2 | C3 | m | m | m | m |
| | C | m | U | HO | HE | S | C1 | C2 | C3 | m | m | m | m |
| | D | m | U | HO | HE | S | C1 | C2 | C3 | m | m | m | m |
| | E | m | U | HO | HE | S | C1 | C2 | C3 | m | m | m | m |
| | F | m | U | HO | HE | S | C1 | C2 | C3 | m | m | m | m |
| | G | m | U | HO | HE | S | C1 | C2 | C3 | m | m | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium but the cells were harvested)
C1 = concanavalin A added to cells (2.5µg/ml)
C2 = concanavalin A added to cells (5.0µg/ml)
C3 = concanavalin A added to cells (10µg/ml)
HO = cells treated with adult homogonic S.ratti excretory/secretory antigen
HE = cells treated with adult heterogonic strain of S.ratti excretory/secretory antigen
S = cells treated with adult S.venezuelensis excretory/secretory antigen

response to the mitogen across the concentration range but rat number 3 had a much lower reponse at a concentrations 5 and 10μg/ml (Fig.9:4b, page 185) which was probably responsible for the highly significant between rat variation ($P<0.001$). Least significant differences between treatment means for each rat showed that 5μg/ml caused the highest level of stimulation for rat 1 ($0.01<P<0.05$), 2.5μg/ml for rat 3 ($0.01<P<0.05$), and there was no difference in the response of rat 2 to any of the concentrations.

Overall, the data from this experiment were strongly influenced by the high levels of activity of non-immune cells from rat B in all treatments, including the unstimulated controls (Fig.9:4a, page 185). This phenomenon completely dominates the differences shown between cells from other rats in response to parasite antigens (Fig.9:4c, page 186). Therefore it could only be concluded that there was no real difference between any of these treatments although, as before, there was a highly significant between rat variation ($P<0.001$).

All worms had been expelled from the infected rats by day 28 post-infection.

### 9.2.3. Expt 3, Dynamics of the cellular response: *S.venezuelensis* priming cell donors

#### 9.2.3.1. Materials and methods

Eighteen rats were divided into three groups:-

Gp.X - 9 rats - infected by skin-application with 2000 third-stage *S.venezuelensis* larvae, to provide immune mesenteric lymph node cells.

Gp.Y - 6 animals - untreated, provided normal mesenteric lymph node

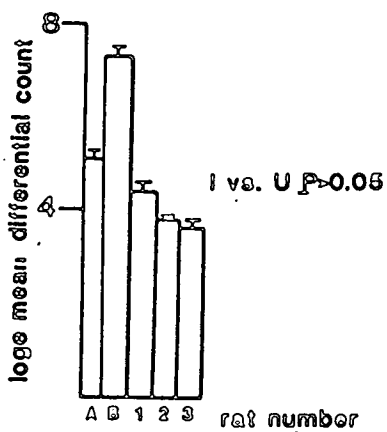
Fig.9:4

The response of cells obtained from donors, either primed with heterogonic strain of *S.ratti* or untreated , to various samples was recorded. The cells were either exposed to medium alone (unstimulated cells, treatment U, Fig.9:4a), concanavalin A (treatment C1-C3, Fig.9:4b) or various worm excretory/secretory antigens (treatments HO, HE and S, Fig.9:4c). Each bar in the histograms refers to results of cells obtained from a particular donor, which was either uninfected (rats A and B) or infected (rats 1-3). The p values shown refer to the overall comparison of the response of cells from uninfected rats (Gp.Y, shown as U) versus infected rats (Gp.X, shown as I) to the various additions.

Fig.9:4

In vitro response of day 28 mesenteric lymph node cells from cell donors, either uninfected or primed with the heterogonic strain of S.ratti, to different preparations, Expt 2

a) Unstimulated activity




b) Concanavalin A stimulation

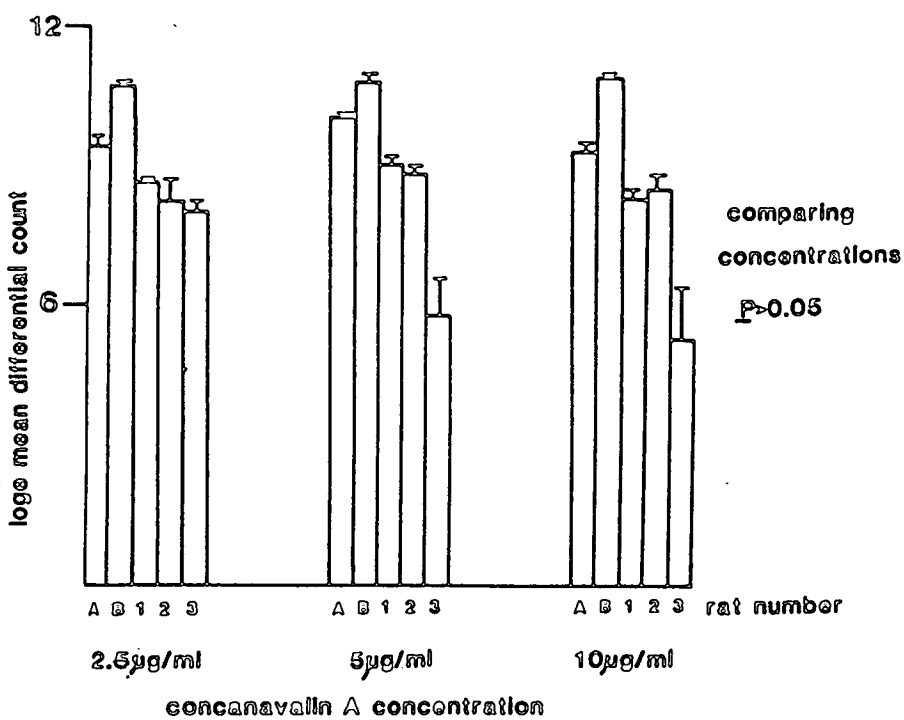
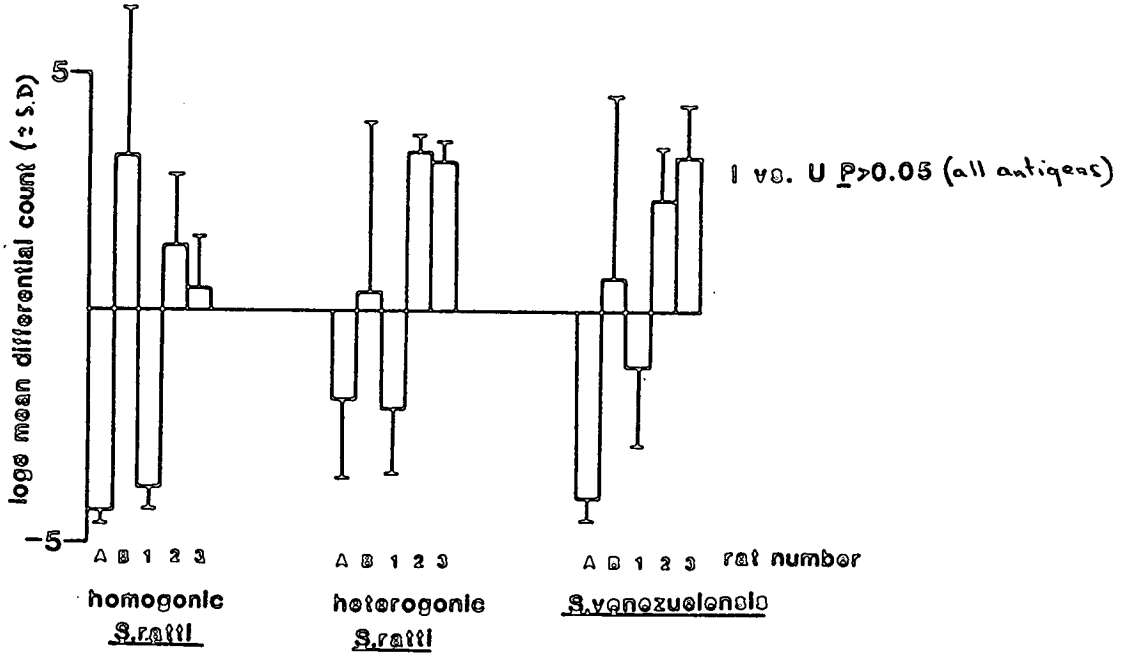


Fig. 9:4 (continued)

c) Cells stimulated with adult excretory/ secretory antigen



cells

Gp.Z - 3 rats - priming controls, infected with an exact dose of <100 infective *S.venezuelensis* larvae in the same way as Gp.X, and killed on day 8 post-infection to give an estimate of the viability of the larvae used at priming.

Three rats from Gp.X and two from Gp.Y were killed on days 8, 12 and 21 post-infection and their mesenteric lymph node cells were used in a lymphocyte transformation assay. The treatments to which the cells were exposed are shown in Fig.9:5, page 189. Worm and egg data from the rats were recorded.

9.2.3.2. Results

On days 8, 12 and 21 post-infection there was no difference in the level of unstimulated activity in normal and immune cells (Fig.9:6a, page 190).

Unfortunately there are no results available for concanavalin A stimulation of cells on days 8 and 12 post-infection since the mitogen used had been stored at a concentration of 7.5µg/ml which seemed to have abolished its effect. A new batch of concanavalin A (sample A) was used on day 21 post-infection and this was highly active compared to the diluted sample (Sample C) used on the previous occasions (Fig.9:6d, page 193). On day 21 there was no significant difference in the level of stimulation in normal and immune cells after treatment with the mitogen but there was a significant between rat variation ($0.001<P<0.01$, Fig.9:6d, page 193).

There was no specific response to any concentration of the homologous adult homogenate or excretory/secretory antigens on day 8 post-infection

(Fig.9:6b(i) and 9:6c(i), pages 190 and 192). However on day 12 post-infection immune cells exhibited a significantly greater response to both adult antigens ($\underline{P}<0.01$, excretory/secretory antigen; $\underline{P}<0.05$, homogenate antigen, Fig.9:6b(ii) and 9:6c(ii), pages 191 and 192). There was no significant difference in the level of stimulation produced in immune cells by the various concentrations of the two antigens nor in their response to the two antigen types (using data for the neat antigens). A similar specific response was demonstrated in day 21 immune cells for both antigens (i.e. $\underline{P}<0.05$, excretory/secretory antigen; $\underline{P}<0.025$, homogenate antigen, Fig.9:6b(iii) and Fig.9:6c(iii), pages 191 and 193). Again varying the concentration of the antigens had no effect ($\underline{P}>0.05$) and a similar level of stimulation was produced by them.

A significantly lower proportion of the dose was recovered from rats infected with 2000 third-stage *S.venezuelensis* larvae compared with those infected with an exact dose of <100 ($\underline{P}<0.01$, Table 8:2(iii), Appendix 8, page 352). There seemed to be a gradual worm loss over the course of infection since there was no significant difference in the proportion of the dose recovered on adjacent days but there was a reduction in the number of eggs *in utero* per worm (Table 8:2(iii), Appendix 8, page 352) suggesting that an anti-worm response was in operation. There was no posterior migration of adults throughout the course of infection, with the majority of worms being recovered from the first section of the intestine (Table 8:3(ii), Appendix 8, page 354).

### 9.3. Homologous vs heterologous challenge

The peak cellular response to the parasites seemed to occur about day 12-16 post-infection since there was an increase in unstimulated activity in

Fig.9:5

Cells from an individual rat were added to all wells of a microtitre plate before the addition of the relevant sample (as shown). Only results of cells in rows B-G of columns 2-9 on days 8 and 12, and of columns 2-11 on day 28 were recorded.

Fig.9:5

Appearance of microtitre plate in Expt 3

i) Days 8 and 12

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | m | m | m |
| | C | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | m | m | m |
| | D | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | m | m | m |
| | E | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | m | m | m |
| | F | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | m | m | m |
| | G | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | m | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

ii) Day 21

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | Cb | Ca | m |
| | C | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | Cb | Ca | m |
| | D | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | Cb | Ca | m |
| | E | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | Cb | Ca | m |
| | F | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | Cb | Ca | m |
| | G | m | U | Cc | A1 | A2 | A3 | H1 | H2 | H3 | Cb | Ca | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium bu
the cells were harvested)
Ca = concanavalin A added to cells (2.5µg/ml, freshly made solution from a
newly recieved batch of powder, Sample A)
Cb = concanavalin A added to cells (2.5µg/ml, solution frozen at a
concentration of 2mg/ml before use, same powder as that used to
prepare Cc, Sample B)
Cc = concanavalin A added to cells (2.5µg/ml, solution frozen at a
concentration of 7.5µg/ml before use, Sample C)
A = cells treated with adult S.venezuelensis excretory/secretory antigen
H = cells treated with adult S.venezuelensis homogenate antigen
1 = antigen used neat
2 = antigen diluted 1:2
3 = antigen diluted 1:4

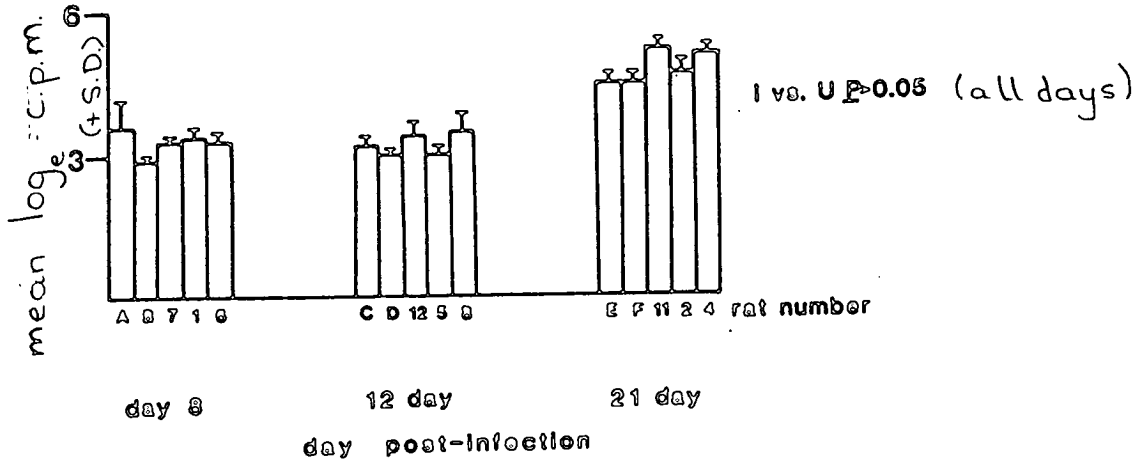
Fig.9:6

The response of cells obtained from donors either infected with *S.venezuelensis* (Gp.X, rats 1–12) or uninfected (Gp.Y, rats A–F) to different preparations is shown. Four main types of samples were tested, medium alone (unstimulated controls, treatment U, Fig.9:6a), homologous worm excretory/secretory antigen (treatment A, Fig.9:6b), homologous worm somatic antigen (treatment H, Fig.9:6c) and mitogen (treatment C, Fig.9:6d). As before, each bar in the figure refers to data collected from one rat and the p values show the results of the response of cells from uninfected versus infected donors (I vs U) to the different treatments. There are also probability levels given which show the overall response of cells from infected donors to different concentrations of the antigens or mitogen.

Fig.9:6

Response of mesentoric lymph node cells to various treatments: S.venezuelensis priming donors of immune cells, Expt 3

a) Unstimulated activity




b) Cells stimulated with adult worm S.venezuelensis excretory/ secretory antigen

i). day 8 post-infection

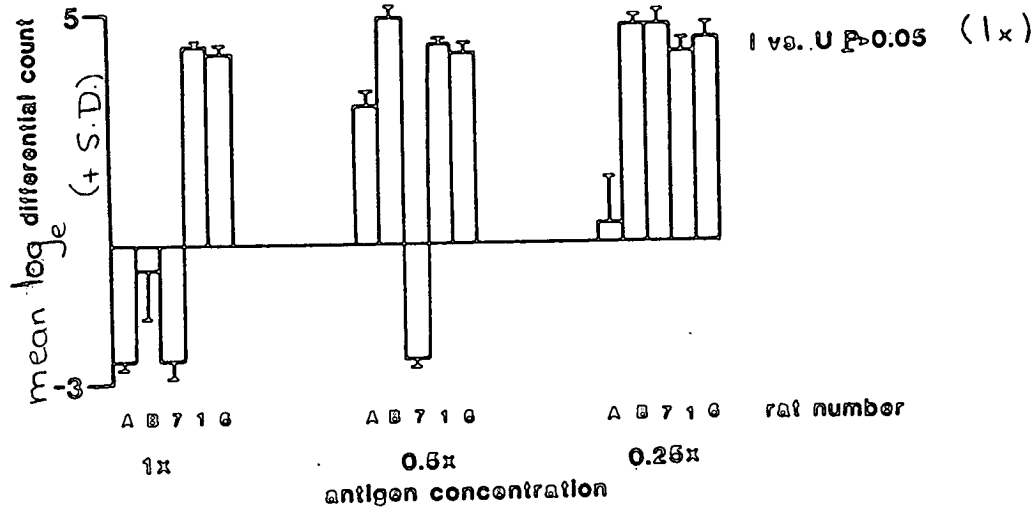
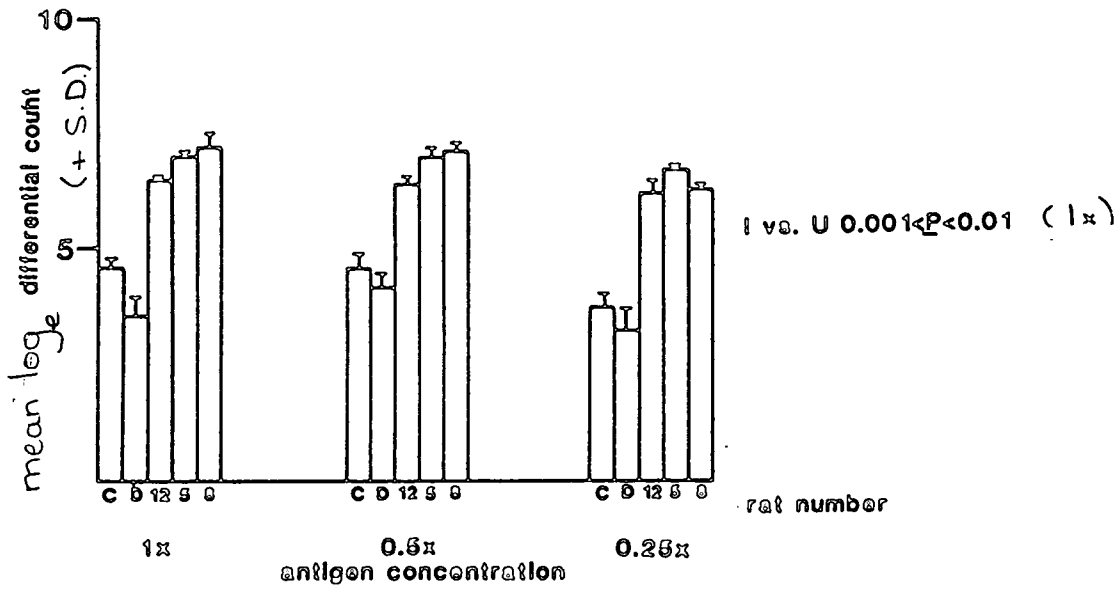


Fig.9:6 (continued)

II) day 12 post-infection




III) day 21 post-infection

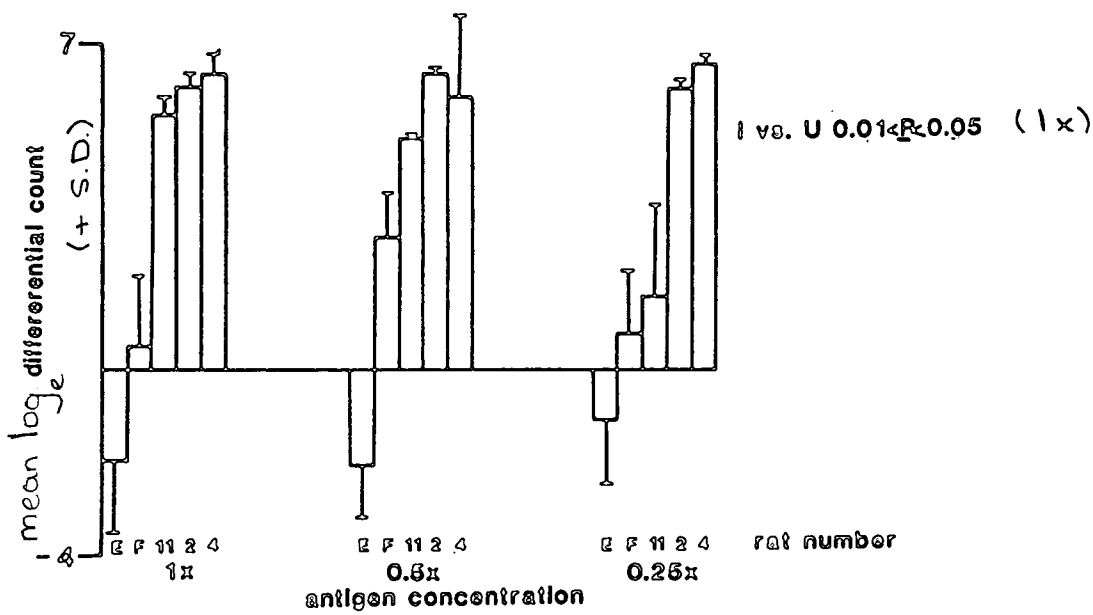
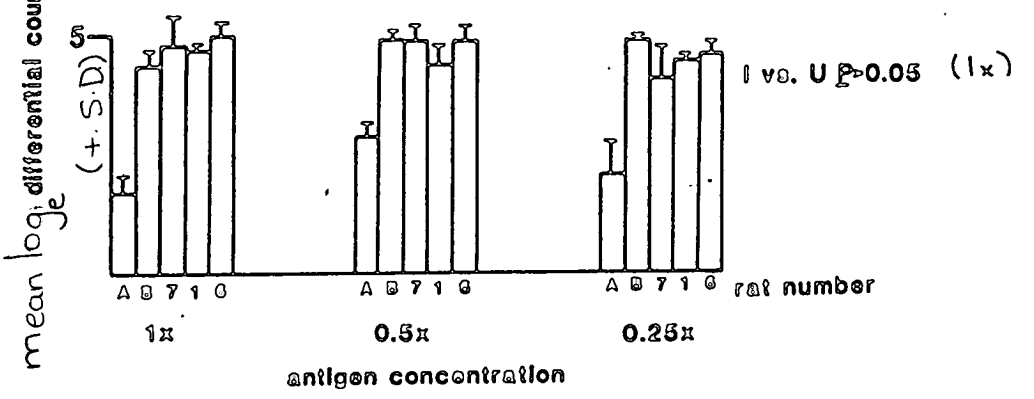


Fig.9:6 (continued)

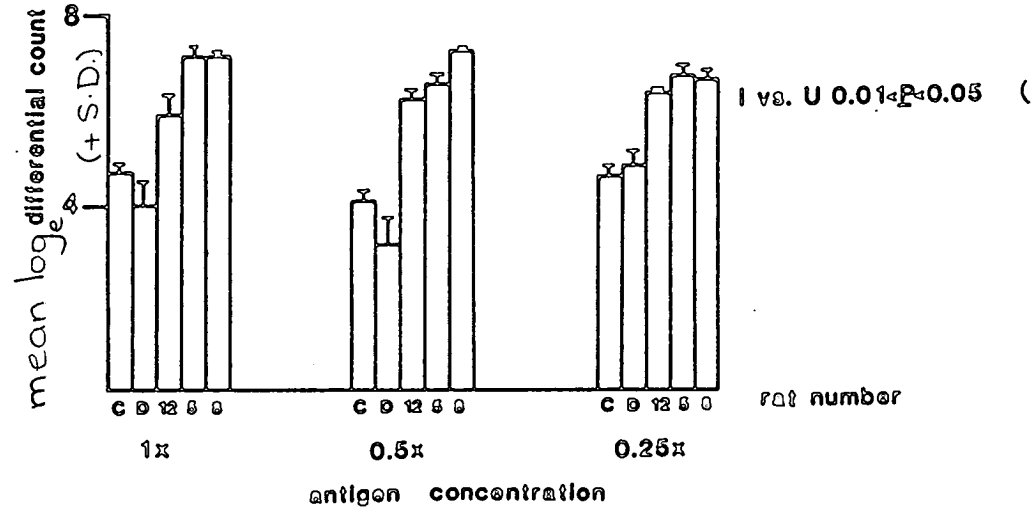
c) Cells stimulated with adult S.venezuelensis worm homogenate antigen

I) day 8 post-infection




I vs. U $P>0.05$ (1x)

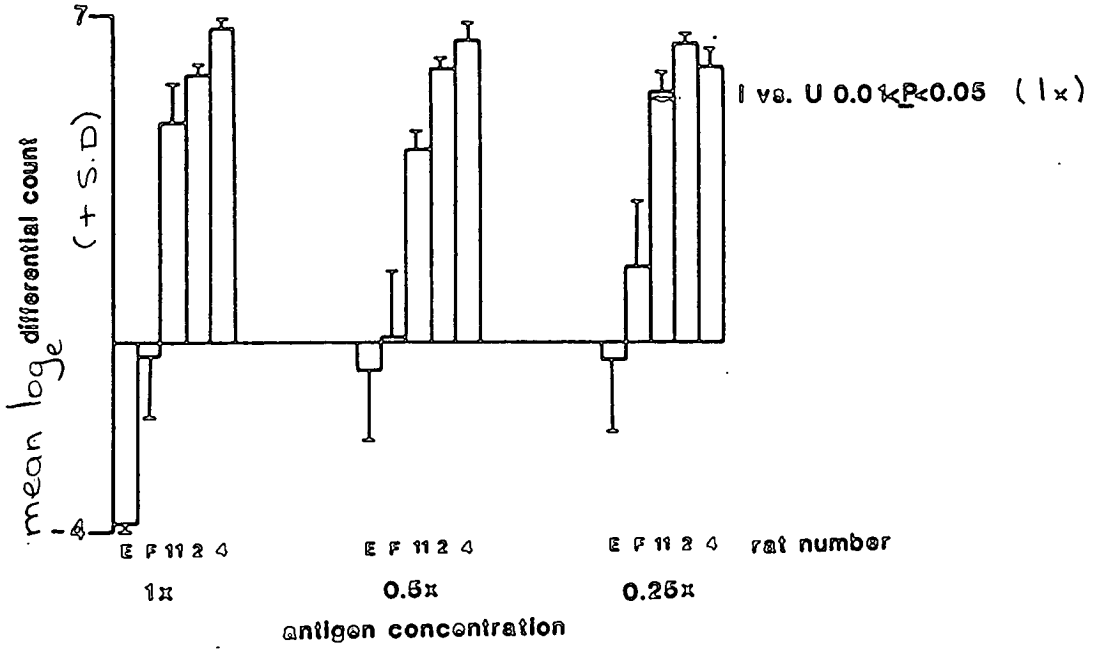
II) day 12 post-infection



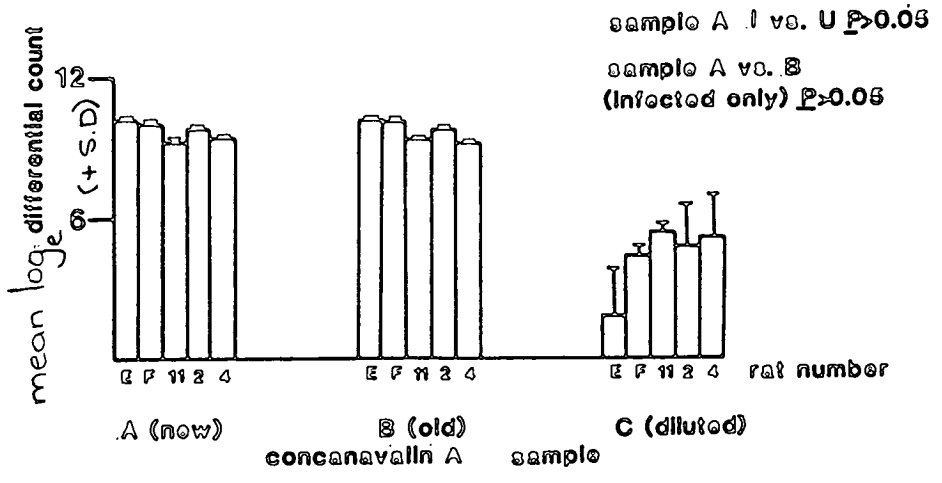

I vs. U $0.01<P<0.05$ (1x)

Fig. 9:6 (continued)

III) day 21 post-infection




d) concanavalin A stimulation day 21 post-infection



immune cells at this time (Expt 1) and a specific response to the antigens (Expt 3). Therefore a number of experiments were conducted using mesenteric lymph node cells obtained on these days to find out if the response to homologous and heterologous challenge varied. In Expts 4-6 antigens produced from adult worms were used, whereas, in Expts 7 and 8, adult and third-stage larval antigens were tested.

### 9.3.1. Expt 4, Homologous vs heterologous challenge: homogonic strain of *S.ratti* priming cell donors

#### 9.3.1.1. Materials and methods

Five rats were divided into 2 groups:- Gp.X - 3 rats - infected by skin-application with 2000 third-stage homogonic *S.ratti* larvae, to provide immune mesenteric lymph node cells.

Gp.Y - 2 rats - uninfected, provided normal cells.

Rats were killed on day 12 post-infection and their cells were plated out as shown in Fig.9:7(i), page 196. The data on worms found in the intestine were recorded as before.

#### 9.3.1.2. Results

There was no significance overall in the difference in the unstimulated activity of cells from normal and immune rats but again, there was a significant between rat variation in the infected group ($P<0.025$). Thus, application of the least significant difference between individual rat means did show that cells from infected rats expressed a higher level of activity than either of the controls ($P<0.05$, Table 9:1a, page 197).

Comparison of the response of the two cell populations to mitogenic stimulation showed that normal cells exhibited a greater response ($P<0.05$, Table 9:1a, page 197) but there was no significant difference in the response of each cell type to the two concanavalin A samples.

The heterologous antigens (neat) produced a specific stimulation of cells from infected rats ($P<0.05$, *S.venezuelensis* antigen; $P<0.01$, heterogonic *S.ratti* antigen, Table 9:1a, page 197) whereas the homologous antigen did not produce specific stimulation overall of immune cells, but there was a significant inter-rat variation ($P<0.05$). Using the least significant difference between means, it was clear that rat 2 had no response, whilst rat 3 had a highly significant response to the antigen ($P<0.001$). Comparison of the response of immune cells from rats 2 and 3 to the two concentrations of the heterologous antigens showed that they produced a similar response to both for the *S.venezuelensis* antigen, but that they deviated in their response to the heterogonic *S.ratti* antigen (in both cases there was a significant difference in the results from rats 2 and 3, $P<0.05$). The least significant difference was not exceeded when comparing individual rats in response to different concentrations of the *S.venezuelensis* antigen, though individual differences were greater than the least significant difference in the case of the heterogonic *S.ratti* antigen, with the higher concentration producing the greater response ($0.01<P<0.05$).

Comparison of the response of immune cells to all three types of adult worm excretory/secretory antigens showed that effects of the three treatments were not additive, since there was a significant interaction detected by the two way analysis of variance (immune rat cells vs antigens $P<0.05$). This interaction was founded on the fact that there was no difference in the

Fig.9:7

Cells from each rat were added to all wells of a single microtitre plate in all three experiments and they were exposed to the appropriate treatment as shown in (i) for Expt 4; (ii) for Expt 5; and (iii) for Expt 6.

Fig.9:7

Appearance of microtitre plate in Expts 4-6

i) Expt 4

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ca | Cb | m | m |
| | C | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ca | Cb | m | m |
| | D | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ca | Cb | m | m |
| | E | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ca | Cb | m | m |
| | F | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ca | Cb | m | m |
| | G | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ca | Cb | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

ii) Expt 5

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | Hb | Ca | P |
| | C | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | Hb | Ca | P |
| | D | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | Hb | Ca | P |
| | E | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | Hb | Ca | P |
| | F | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | Hb | Ca | P |
| | G | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | Hb | Ca | P |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

iii) Expt 6

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | P | Ca | Q |
| | C | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | P | Ca | Q |
| | D | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | P | Ca | Q |
| | E | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | P | Ca | Q |
| | F | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | P | Ca | Q |
| | G | m | U | HO1 | HO2 | HE1 | HE2 | S1 | S2 | Ha | P | Ca | Q |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium but the cells were harvested)
Ca = concanavalin A added to cells (2.5µg/ml, freshly made)
Cb = concanavalin A added to cells (2.5µg/ml, solution frozen at a concentration of 2mg/ml before use)
HO = adult homogonic strain of S.ratti excretory/secretory antigen added to cells
HE = adult heterogonic strain of S.ratti excretory/secretory antigen added to cells
S = adult S.venezuelensis excretory/secretory antigen added to cells
1 = antigen used neat
2 = antigen diluted 1:2
Ha = adult heterogonic strain of S.ratti homogenate antigen added to cells, stored overnight at +4°C before use, used neat
Hb = adult heterogonic strain of S.ratti homogenate antigen added to cells, stored at -20°C overnight before use, used neat, same batch as Ha
P = combination of Ca + Ha added to cells (concentration of the mitogen was adjusted to remain at 2.5µg/ml but the antigen was diluted 1:2)
Q = combination of Ca + HE added to cells (concentration of the mitogen was adjusted to remain at 2.5µg/ml but the antigen was diluted 1:2)

Table 9:1

Results for mesenteric lymph node cells in Expts 4–6, which were obtained from either uninfected (A and B), or infected (1–3), donors. The effect of the mitogen treatments only are shown for Expt 6 (iii) since there was no specific response to the other treatments but their data can be found in Table 8:1(ix) Appendix 8, page 348

Table 9:1

Homologous vs heterologous challenge, results of Expts 4, 5 and 6, data $\log_e$ transformed

a) Expt 4, homogenic strain of S.rotti priming cell donors

| Rat no. | | Treatment U | Co | Cb | KO2 | KO1 | HE2 | HE1 | S2 | S1 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Uninfected** | | | | | | | | | | |
| A | mean | 4.36 | 9.70 | 9.64 | -0.07 | -0.54 | -1.17 | -1.14 | -1.65 | -0.05 |
| | SD | 0.50 | 0.13 | 0.15 | 2.77 | 3.40 | 3.60 | 3.09 | 3.40 | 4.27 |
| | n | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| B | mean | 4.54 | 10.08 | 10.16 | -2.63 | 0.72 | 0.65 | -0.93 | -1.43 | 1.02 |
| | SD | 0.33 | 0.09 | 0.14 | 2.33 | 3.31 | 3.01 | 2.40 | 3.27 | 2.75 |
| | n | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| **Infected** | | | | | | | | | | |
| 2 | mean | 6.01 | 7.99 | 8.60 | -1.63 | -1.59 | 4.35 | 7.00 | 5.09 | 6.20 |
| | SD | 0.66 | 1.53 | 0.09 | 4.62 | 5.67 | 4.05 | 0.52 | 0.84 | 0.83 |
| | n | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| 3 | mean | 5.07 | 8.47 | 8.96 | 0.17 | 4.54 | 5.15 | 6.07 | 5.06 | 5.36 |
| | SD | 0.16 | 0.76 | 0.23 | 4.45 | 1.08 | 0.55 | 0.97 | 1.09 | 1.04 |
| | n | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |

b) Expt 5, heterogenic strain of S.rotti priming cell donors

| Rat no. | | Treatment U | Co | KO2 | KO1 | HE2 | HE1 | S2 | S1 | Ha | Hb | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Uninfected** | | | | | | | | | | | | |
| A | mean | 5.39 | 9.86 | 4.34 | -0.97 | 0.05 | -0.60 | 2.70 | -1.80 | 1.37 | 5.03 | 9.09 |
| | SD | 0.32 | 0.33 | 1.24 | 5.13 | 5.23 | 5.49 | 5.29 | 4.17 | 5.32 | 1.11 | 0.21 |
| | n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 5 |
| B | mean | 6.24 | 8.39 | -0.35 | 1.86 | 5.16 | 0.95 | 1.88 | 0.56 | 0.75 | 1.30 | 7.94 |
| | SD | 1.23 | 0.21 | 6.29 | 5.93 | 3.84 | 5.77 | 5.72 | 6.57 | 5.71 | 5.75 | 0.43 |
| | n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 5 |
| **Infected** | | | | | | | | | | | | |
| 1 | mean | 8.40 | 7.66 | 8.77 | 6.83 | 7.95 | 7.80 | 5.71 | 8.48 | 8.17 | 8.41 | 2.26 |
| | SD | 0.59 | 0.31 | 0.15 | 7.97 | 0.70 | 0.90 | 5.81 | 0.46 | 0.90 | 0.76 | 7.87 |
| | n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 5 |
| 2 | mean | 8.43 | 7.31 | 4.43 | 9.14 | 5.23 | 8.53 | 7.57 | 7.91 | 8.37 | 8.38 | -7.14 |
| | SD | 0.84 | 0.60 | 6.75 | 0.38 | 7.58 | 0.58 | 0.78 | 0.99 | 0.65 | 0.68 | 1.61 |
| | n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 5 |
| 3 | mean | 8.13 | 9.12 | 7.09 | 5.20 | 3.17 | 2.17 | 2.14 | 0.68 | 8.56 | 4.40 | 8.58 |
| | SD | 0.58 | 0.48 | 0.92 | 5.78 | 5.39 | 7.74 | 7.72 | 7.22 | 0.62 | 5.91 | 0.67 |

c) Expt 6, S.venezuelensis priming cell donors

| Rat No. | | Treatment U | Co | Q | P |
|---|---|---|---|---|---|
| **Uninfected** | | | | | |
| A | mean | 5.52 | 7.74 | 7.17 | 8.04 |
| | SD | 0.46 | 0.34 | 0.23 | 0.22 |
| | n | 5 | 5 | 5 | 5 |
| B | mean | 4.84 | 8.51 | 8.39 | 8.39 |
| | SD | 0.43 | 0.25 | 0.17 | 0.17 |
| | n | 5 | 5 | 5 | 5 |
| **Infected** | | | | | |
| 1 | mean | 7.46 | 6.88 | 8.40 | 8.43 |
| | SD | 0.37 | 8.64 | 0.31 | 0.31 |
| | n | 5 | 5 | 5 | 5 |
| 2 | mean | 7.07 | -3.19 | 8.60 | 5.21 |
| | SD | 0.93 | 5.56 | 7.25 | 5.86 |
| | n | 5 | 5 | 5 | 5 |
| 3 | mean | 7.69 | 8.90 | 8.99 | 8.49 |
| | SD | 0.66 | 0.13 | 0.17 | 0.27 |
| | n | 5 | 5 | 5 | 5 |

Where:-

U = unstimulated control treatment
Co = cells treated with concanavalin A (2.5µg/ml, freshly made)
Cb = cells stimulated with concanavalin A (2.5µg/ml, solution frozen at a concentration of 2mg/ml before use)
KO = homogenic strain of S.rotti adult excretory/secretory antigen added to cells
HE = heterogenic strain of S.rotti adult excretory/secretory antigen added to cells
S = S.venezuelensis adult excretory/secretory antigen added to cells
1 = antigen used neat
2 = antigen diluted 1:2
Ha = heterogenic strain of S.rotti adult homogenate antigen added to cells, stored overnight at +4°C, used neat
Hb = heterogenic strain of S.rotti adult homogenate antigen added to cells, stored overnight at -20°C before use, used neat
P = combination of Co + Ha added to cells (concentration of the antigen was adjusted to remain at 2.5µg/ml, but the antigen was diluted 1:2)
Q = combination of Co + HE added to cells (concentration of the antigen was adjusted to remain at 2.5µg/ml, but the antigen was diluted 1:2)

response of rat 3 to all three antigens, whereas, while rat 2 exhibited no response to the homogonic *S.ratti* antigen, it had a positive response to the other two (Table 9:1a, page 197).

### 9.3.2. Expt 5, Homologous vs heterologous challenge: heterogonic strain of *S.ratti* priming cell donors

#### 9.3.2.1. Materials and methods

Five rats were divided into two groups:-

Gp.X - 3 rats - infected by skin-application with 2000 infective heterogonic strain *S.ratti* larvae, provided immune mesenteric lymph node cells.

Gp.Y - 2 rats - untreated, provided non-immune cells.

All the rats were killed on day 12 of Gp.X's infection and their cells were used in a transformation assay (see Fig.9:7(ii), page 196). Egg and worm data were collected from the rats.

#### 9.3.2.2. Results

Immune cells expressed a significantly higher level of unstimulated activity compared with normal cells ($0.001<P<0.01$, Table 9:1(b), page 197) but there was no difference in the level of mitogen-induced blastogenesis of the two cell populations, although there was a significant between rat variation within treatments ($P<0.001$, Table 9:1b, page 197).

The response to antigenic stimulation was variable (Table 9:1b, page 197). A positive specific response was induced by heterologous challenge with the

homogonic *S.ratti* excretory/secretory worm antigen ($P<0.05$) but challenge with similar preparations from *S.venezuelensis* or the homologous heterogonic *S.ratti* worms produced no effect. A positive response was however, produced by the homologous heterogonic *S.ratti* homogenate antigen ($P<0.001$). Inspection of the results (Table 9:1b, page 197) shows that the results from rat 3 probably had a strong influence on the overall outcome of any analysis since its cells expressed a very depressed response to two of the three adult worm excretory/secretory antigens compared with those from rats 1 and 2.

There was no difference in the response of cells from infected rats to treatment with the two samples of heterogonic *S.ratti* homogenate antigen suggesting that freezing does not affect the ability of the antigenic preparation to stimulate cells. Treatment of cells with a mixture of heterogonic *S.ratti* homogenate antigen and concanavalin A did not increase the level of stimulation of the immune cells above that already obtained after treatment with mitogen only (Table 9:1b, page 197).

### 9.3.3. Expt 6, Homologous vs heterologous Challenge: *S.venezuelensis* priming cell donors

#### 9.3.3.1. Materials and methods

Five rats were divided into two groups:-

Gp.X - 3 rats - infected by skin-application with 2000 infective *S.venezuelensis* larvae, provided immune mesenteric lymph node cells.

Gp.Y - 2 rats - uninfected, provided normal cells

On day 12 of the infection animals in both groups were killed and their

cells were used in an assay to assess their response to various treatments (Fig.9:7(iii), page 196). Egg and worm data were also collected.

9.3.3.2. Results

There was a significantly greater level of unstimulated activity obtained in immune cells ($\underline{P}<0.01$, Table 9:1c, page 197) but the response of the two cell populations to mitogenic stimulation was similar, although there was a significant between rat variation ($\underline{P}<0.001$). There was no response to any antigen preparation (Table 8:1(ix), Appendix 8, page 348) and comparison of the results obtained from immune cells treated with concanavalin A on its own versus mixtures of concanavalin A with either heterogonic excretory/secretory or homogenate worm antigen, showed there was no significant difference in the level of stimulation produced (Table 9:1c, page 197).

9.3.3.3. Results of worm data for Expts 4–6

Comparison of the worm burdens obtained on day 12 post-infection in experiments 4, 5 and 6 showed there was a significant difference in the results ($\underline{P}<0.001$, Tables 8:2(iv)-(vi), Appendix 8, page 352). Application of least significant differences between treatment means demonstrated that the highest proportion of the dose was recovered from rats infected with the homogonic strain of *S.ratti* ($\underline{P}<0.05$, compared to heterogonic strain of *S.ratti*, $\underline{P}<0.001$, compared with *S.venezuelensis*). The lowest proportion of the dose was recovered from rats infected with *S.venezuelensis* ($\underline{P}<0.01$, compared with both *S.ratti* strains).

### 9.3.4. Expts 7 and 8, Homologous vs heterologous challenge, adult and third-stage larval antigens: heterogonic *S.ratti* priming cell donors

#### 9.3.4.1. Materials and methods

Essentially the same protocol was used in both experiments. Ten rats were divided into 2 groups:-

Gp.X - 6 rats - infected by skin-application with 2000 infective heterogonic strain *S.ratti* larvae, provided immune mesenteric lymph node cells.

Gp.Y - 4 rats - uninfected, provided normal cells.

Three rats from Gp.X and two from Gp.Y were killed on days 12 and 16 post-infection, their cells were plated out as shown in Fig.9:8(i), Expt 7, and Fig.9:8(ii), Expt 8, page 203. Egg and worm data were recorded for each rat.

#### 9.3.4.2. Results

There was no difference in the unstimulated activity of normal and immune cells in Expt 7 on days 12 and 16 post-infection, but there was a significant between rat variation within treatments in both cases ($P<0.001$, Fig.9:9a, page 204). Calculation of the least significant difference between individual rat means at day 12 and day 16 showed that immune cells demonstrated a higher level of stimulation ($P(0.05$, day 12; $P<0.001$, day 16) which could have been masked by the between rat variation. However, in Expt 8 immune cells exhibited a significantly greater level of overall unstimulated activity on day 12 post-infection ($P<0.05$, Fig.9:10a, page 207) despite a significant between rat variation within treatments ($P<0.001$). A similar higher activity in immune

cells was not demonstrated on day 16 post-infection in Expt 8 (Fig.9:10a, page 207).

Initial analysis showed there was no difference in the level of mitogen-induced stimultion exhibited by normal and day 12 immune cells in Expt 7 but it was obvious from the data that the results from rat 1 were affecting the outcome since cells from rats 2 and 5 had a depressed response (Fig.9:9f, page 206, results for stimulation with sample A of concanavalin A only are shown for days 12 and 16 since there was no significant difference in the response of individual rat cells to both). Day 16 immune cells from Expt 7 express a significantly lower level of blastogeneseis ($\underline{P}<0.001$) despite the fact that there was, as usual, a significant between rat variation within treatments ($\underline{P}<0.001$). In Expt 8 there was no difference in the level of mitogen-induced blastogenesis of cells from infected and uninfected rats on both day 12 and 16 post-infection (Fig.9:10b, page 207). These results are confused by the anomalous results of rat 4.

In Expt 7 none of the adult excretory/secretory antigens produced significant specific stimulation of day 12 immune cells (Fig.9:9b, page 204) whereas the *S.ratti* larval antigens did ($\underline{P}<0.01$, Fig.9:9d, page . 205) but there was also a significant between rat variation within treatments ($\underline{P}<0.001$). Direct comparison of the effect of antigens from larvae and adult *S.ratti* confirmed that the larval antigens were more potent in this particular assay ($\underline{P}<0.001$). Consistent with results from day 12 cells, none of the adult excretory/secretory antigens caused specific stimulation of those from day 16, but inspectation of the data shows that overall, the means for individual rats were higher for infected animals, but that the high variation in the data was probably masking any effect (Fig.9:9c, page 205). There was no significant

Fig.9:8

Cells from an each rat were dispensed into a separate microtitre plate in both experiments and the appropriate sample added to each well as shown in (i) for Expt 7 and (ii) for Expt 8. Because of doubts about the potency of the concanavalin A in previous assays, 2 different sources of the mitogen were used in experiment 7.

Fig.9:8

Appearance of microtitre plate in Expts 7 and 8

i) Expt 7

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | HE | HO | S | HEL | HOL | SL | Ca | Cb | m | m |
| | C | m | U | HE | HO | S | HEL | HOL | SL | Ca | Cb | m | m |
| | D | m | U | HE | HO | S | HEL | HOL | SL | Ca | Cb | m | m |
| | E | m | U | HE | HO | S | HEL | HOL | SL | Ca | Cb | m | m |
| | F | m | U | HE | HO | S | HEL | HOL | SL | Ca | Cb | m | m |
| | G | m | U | HE | HO | S | HEL | HOL | SL | Ca | Cb | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

ii) Expt 8

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U | HE | HO | S | HEL | HOL | Ca | m | m | m | m |
| | C | m | U | HE | HO | S | HEL | HOL | Ca | m | m | m | m |
| | D | m | U | HE | HO | S | HEL | HOL | Ca | m | m | m | m |
| | E | m | U | HE | HO | S | HEL | HOL | Ca | m | m | m | m |
| | F | m | U | HE | HO | S | HEL | HOL | Ca | m | m | m | m |
| | G | m | U | HE | HO | S | HEL | HOL | Ca | m | m | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium but the cells were harvested)
Ca = concanavalin A added to cells (2.5µg/ml, freshly made from the powder)
Cb = concanavalin A added to cells (2.5µg/ml, solution frozen at a concentration of 2mg/ml before use)
HE = cells treated with adult heterogonic strain of S.ratti excretory/secretory antigen
HO = cells treated with adult homogonic strain of S.ratti excretory/secretory antigen
S = cells treated with adult S.venezuelensis excretory/secretory antigen
L = larval homogenate antigen added to cells (of the species or strain shown, e.g. SL, S.venezuelensis larval homogenate antigen used)

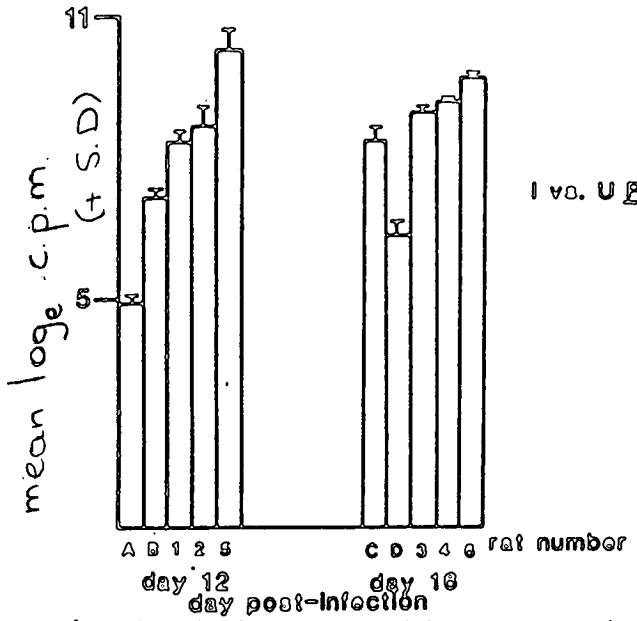
Fig.9:9

The response of cells obtained from uninfected (rats A-D) and infected donors (rats 1-6) to four main treatments is shown. That is, addition of medium alone (unstimulated controls, treatment U, Fig.9:9a), homologous and heterologous worm excretory/secretory antigens (treatments HE, HO and S, Fig.9:9b and Fig.9:9c), homologous and heterologous somatic third-stage larval antigens (treatments HEL, HOL and SL, Fig.9:9d and Fig.9:9e) and concanavalin A (treatment Ca,Fig.9:9f). As before, the P values show the results of the overall comparions and each bar shows the results of cells from one rat.

Fig. 9:9

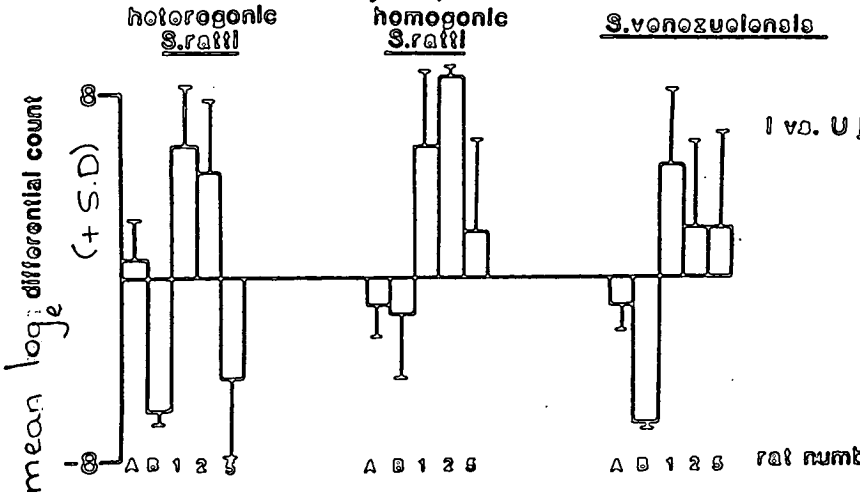
In vitro response of normal and immune cells to different treatments: heterogenic strain of S.ratti used to prime donors of immune cells. Expt 7

a) Unstimulated activity




1 vs. U $P>0.05$ (days 12 and 16)

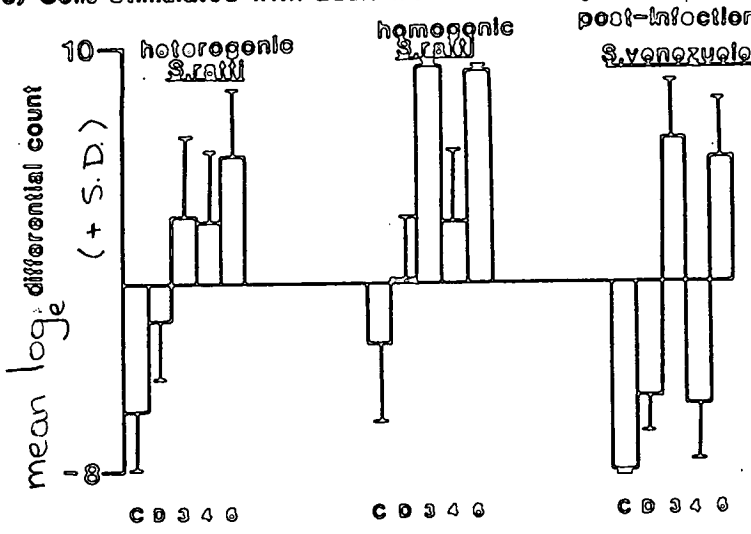
b) Cells stimulated with adult worm excretory/ secretory antigen day 12 post-infection




1 vs. U $P>0.05$ (all antigen

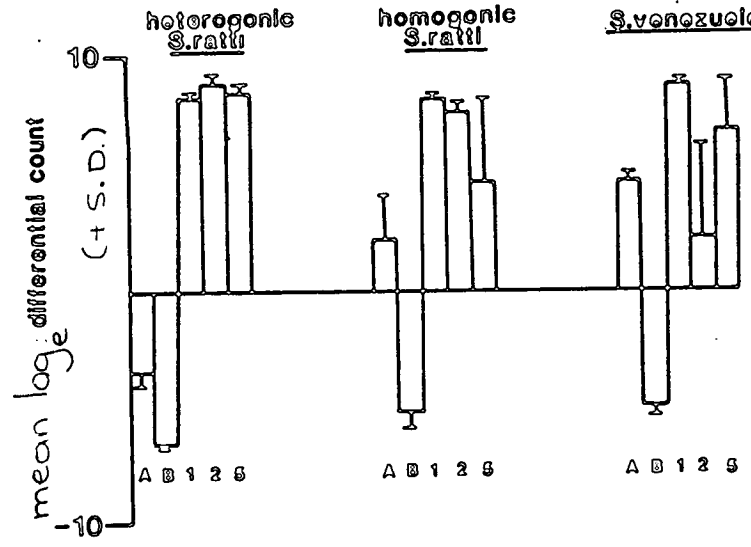
Fig. 9:9 (continued)

c) Cells stimulated with adult worm excretory/ secretory antigen day 18 post-infection




1 vs. U $P>0.05$ (heterogonic antigen)

d) Cells stimulated with larval antigens day 12 post-infection



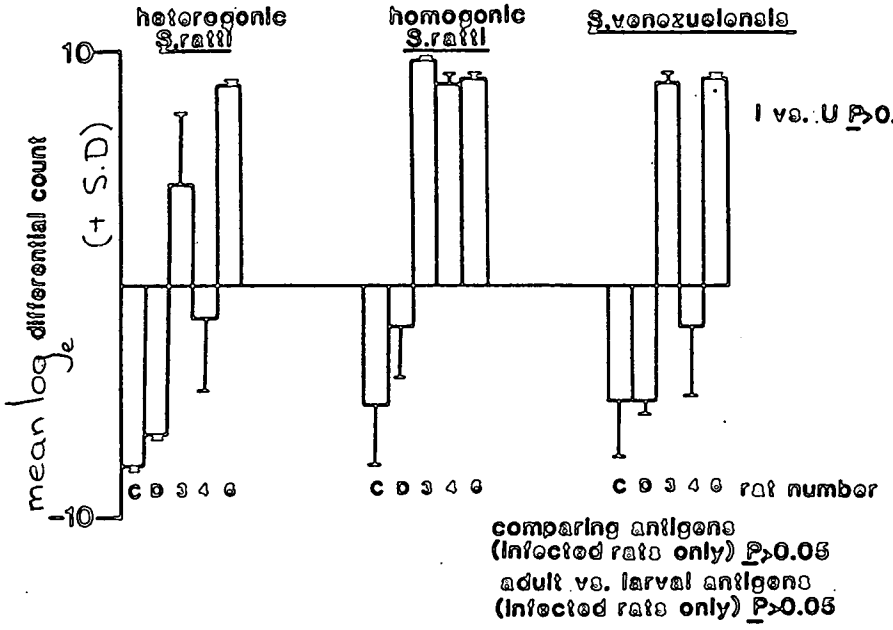

1 vs. U $0.001<P<0.01$ (heterog antige

comparing larval antigens (infected rats only) $P>0.05$

comparing adult vs. larval antigens (infected rats only) $0.01 P>0.05$

Fig. 9:9 (continued)

e) Cells stimulated with larval antigens day 16 post-infection




f) Cells stimulated with concanavalin A

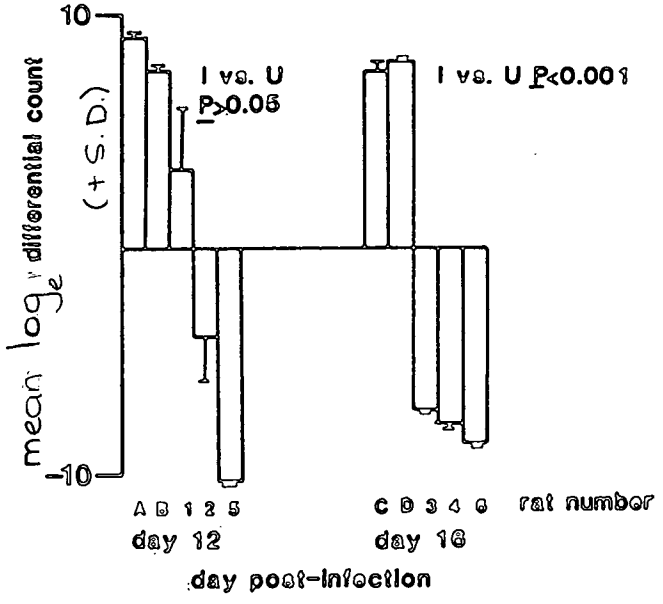


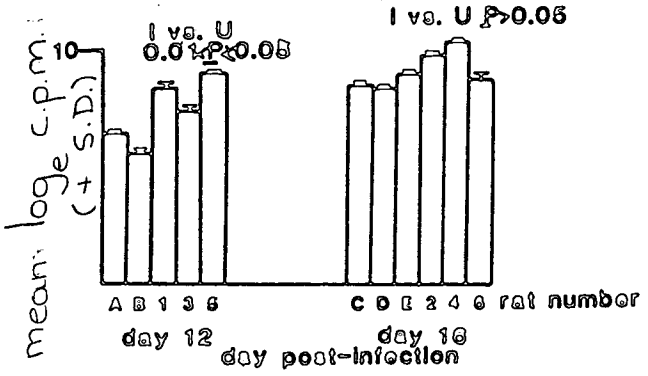
Fig.9:10

The effect of stimulation of cells obtained from uninfected (rats A-E) and infected donors (rats 1-6) with various preparations is shown. Namely, addition of medium alone (unstimulated controls, treatment U, Fig.9:10a), concanavalin A (treatment Ca, Fig.9:10b), homologous and heterologous worm excretory/secretory antigens (treatments HE, HO and S, Fig.9:10c and Fig.9:10d) and *S.ratti* third-stage somatic antigens (treatments HEL and HOL, Fig.9:10e and Fig.9:10f). The probability values given show the results of overall comparisons and each bar in the histograms shows the results of one rat.

Fig. 9:10

Stimulation with various samples: heterogenic strain of S. rotti used to prime donors of immune mesenteric lymph node cells. Expt 8

a) Unstimulated activity




b) Cells stimulated with concanavalin A

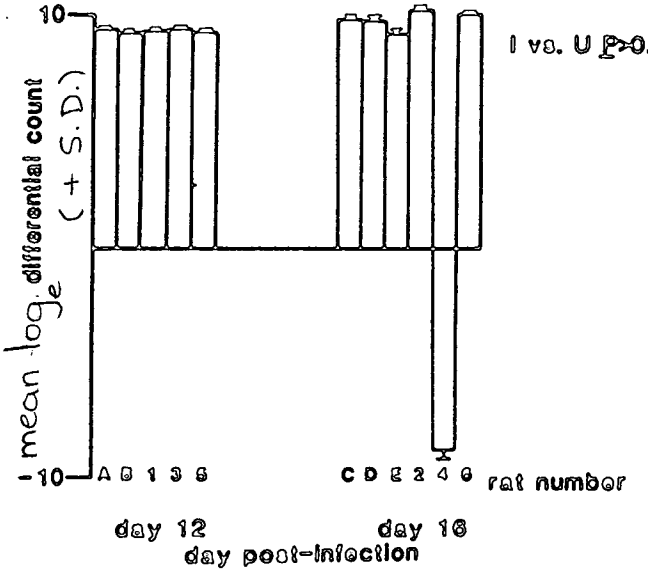
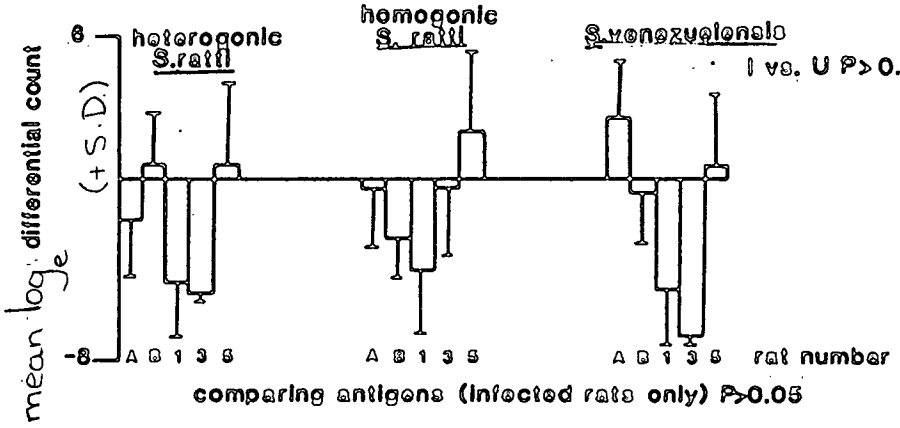


Fig. 9:10 (continued)

c) adult worm excretory/ secretory antigen day 12 post-infection




d) adult worm excretory/ secretory antigen day 16 post-infection

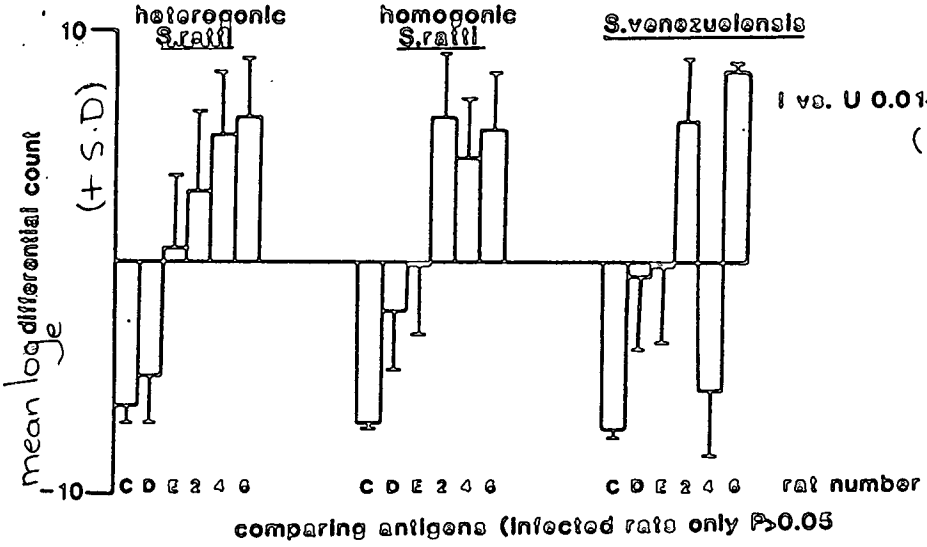
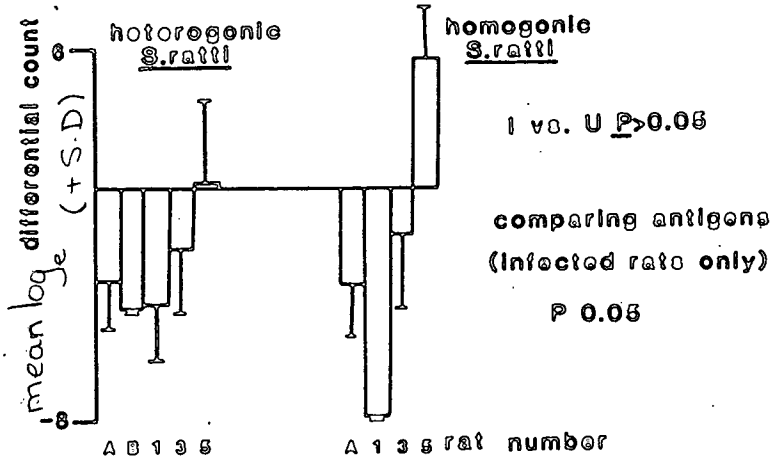
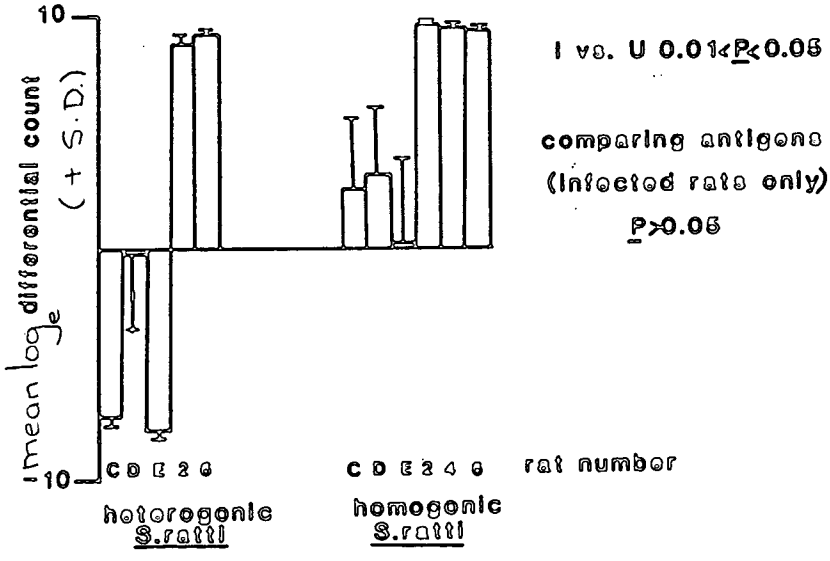


Fig. 9:10 (continued)

e) Cells stimulated with larval antigens day 12 post-infection




f) Cells stimulated with larval antigens day 16 post-infection



specific stimulation by the heterogonic *S.ratti* and *S.venezuelensis* larval antigens on day 16 but the homogonic *S.ratti* antigen had an effect (Fig.9:9e, page 206).

In Expt 8 none of the antigen preparations caused a significant specific stimulation of day 12 cells from two of the three infected rats (Fig.9:10c and 9:10e, pages 208 and 209). With regard to day 16 and adult antigens, the data at first sight (Fig.9:10d and Fig.9:10f, pages 208 and 209) indicated that the response of cells from infected rats was not uniform across antigen types. Two-way analysis of variance confirmed this to be the case, the interaction between individual rats on the one hand, and parasite antigens on the other, being significant at $P<0.05$. A series of one-way analysis of the same data showed that cells responded in a consistent pattern to *S.ratti* antigens, but rat 4 behaved anomolously with respect to *S.venezuelensis* antigen. Treatment with larval antigens produced a specific response in day 16 immune cells ($P<0.05$) which was similar for homologous and heterologous challenge. Comparison of the level of blastogenesis produced by adult and larval antigens in immune cells showed that both were equally ineffective (day 12) or effective (day 16), bearing in mind that *S.venezuelensis* antigens were not tested.

The worm burdens obtained in experiments 7 and 8 were compared using a two-way analysis of variance. There was no significant difference in the proportion of the dose recovered on days 12 or 16 for either experiment, but a significantly lower proportion of the dose recovered on day 16 compared with day 12 post-infection ($P<0.05$, Table 8:2(vii) and (viii), Appendix 8, page 353 ). There was a possible posterior migration of adults on day 16 compared with day 12 post-infection in both experiments (Table 8:3(vi), Appendix 8, page 355).

## 9.4. Effect of cell density on the amount of stimulation produced [Expt 9]

From the foregoing it can be seen that specific responses to parasite antigens of cells from primed rats did occur under some conditions. However, throughout these assays, it was impossible to demonstrate the difference between homologous and heterologous challenge that might have been expected from the present studies of cross resistance and adoptive immunity (Chapters 5 and 8). Several features of the experimental system may have made it insensitive to detect a difference, supposing one to exist. One obvious test of the method was to see whether it could discriminate between different numbers of cells responding, since the assumption being made was that it could.

### 1. Materials and methods

Cell suspensions were prepared from the mesenteric lymph nodes of three uninfected rats and the cell concentrations estimated. Aliquots of the original cell suspension from each rat were made up to 10ml with cell medium to give a final concentration of $1x10^6$, $2x10^6$ and $4x10^6$ cells/ml. These cells were plated out as shown in Fig.9:11, page 212, and one plate was prepared for each rat. The cells were cultured as described in Chapter 2, Section 2:6, page 38, with addition of tritiated-thymidine on day 3. Seventeen hours later the cells were harvested and the level of radio-activity present per treatment found.

### 2. Results

There was a significant difference in the pattern of response of individual

Fig.9:11

Appearance of microtitre plate in Expt 9

| Column no. | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Row no. | A | m | m | m | m | m | m | m | m | m | m | m | m |
| | B | m | U1 | C1 | C1 | U2 | C2 | C2 | U3 | C3 | C3 | m | m |
| | C | m | U1 | C1 | C1 | U2 | C2 | C2 | U3 | C3 | C3 | m | m |
| | D | m | U1 | C1 | C1 | U2 | C2 | C2 | U3 | C3 | C3 | m | m |
| | E | m | U1 | C1 | C1 | U2 | C2 | C2 | U3 | C3 | C3 | m | m |
| | F | m | U1 | C1 | C1 | U2 | C2 | C2 | U3 | C3 | C3 | m | m |
| | G | m | U1 | C1 | C1 | U2 | U2 | C2 | U3 | C3 | C3 | m | m |
| | H | m | m | m | m | m | m | m | m | m | m | m | m |

Where:-

m = medium only added to the cells which were not harvested
U = unstimulated control treatment (same treatment as cells with medium but the cells were harvested)
C = concanavalin A added to cells ($2\frac{5}{6}$ug/ml)
1 = cells at a concentration of $1x10^5$/ml
2 = cells at a concentration of $2x10^5$/ml
3 = cells at a concentration of $4x10^5$/ml

rats to mitogenic stimulation over the range of cell densities, since a significant interaction was present in the results ($P<0.001$, Table 9:2, page 214). A series of one-way analyses of variance showed that cells of rat number 3 expressed a similar level of blastogenesis at each cell concentration. Those of rat number 1 exhibited the highest level of triated-thymidine incorporation at a density of $1x10^5$ and the lowest at $4x10^5$cells/well. There was no difference in the proliferation of cells from rat number 2 incubated at a concentration of $1x10^5$ or $2x10^5$cells/well, but incubation at a concentration of $4x10^5$ produced a lower response. Overall the level of unstimulated blastogenesis obtained at all cell concentrations seemed to be the same ($P>0.05$), although there was a significant between rat variation ($P<0.025$, Table 9:2, page 214).

### 3. Common techniques used througout all experiments

The methods used to infect rats, to count the number of adults present, and the number of eggs *in utero* per worm throughout these experiments are described in Chapter 2, Section 2:10, page 47. The method of assessing the level of radio-activity present is described in Chapter 2, Section 2:6, page 38 and the method of analysing the data is given in Section 2:11:3, page 53. The antigen pools used in each experiment are given in Appendix 8, Section 8:4, page 356. Actual results obtained in this section are given in Tables 8:1(i)-(xv), Appendix 8, pages 344-364.

Table 9:2

Effect of varying cell density, Expt 9 ( data $\log_e$ transformed)

| Rat no. | | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | U3 | C3 | U2 | C2 | U1 | C1 |
| 1 | mean | 5.29 | 8.34 | 4.80 | 9.27 | 4.87 | 9.55 |
| | SD | 0.65 | 0.32 | 0.30 | 0.33 | 0.35 | 0.18 |
| | n | 6 | 10 | 6 | 10 | 6 | 11 |
| 2 | mean | 5.23 | 8.39 | 4.59 | 8.52 | 5.26 | 9.04 |
| | SD | 0.72 | 0.63 | 0.30 | 1.29 | 0.56 | 0.22 |
| | n | 6 | 11 | 6 | 11 | 6 | 11 |
| 3 | mean | 4.99 | 9.08 | 4.84 | 9.50 | 5.51 | 9.20 |
| | SD | 0.33 | 0.38 | 0.31 | 0.15 | 0.28 | 0.16 |
| | n | 6 | 11 | 6 | 11 | 6 | 10 |

Where:-

U = unstimulated control treatment
C = cells treated with concanavalin A (2.5ug/ml)
1 = cell density of $1x10^5$ cells/ml
2 = cell density of $2x10^5$ cells/ml
3 = cell density of $4x10^5$ cells/ml

## 9.5. Summary

a) A higher level of unstimulated blastogenesis occurred in immune cells taken from rats at days 12-16 of an with *Strongyloides spp*infection compared with their normal counterparts. Early in infection (day 8), there was no consistent increase in increased activity in immune cells though individual rats showed the effect. By day 21-28 post-infection the disparity had disappeared.

b) The response of cells taken during the later stages of infection with *Strongyloides spp.* from day 12 onwards, showed a depressed response to mitogenic stimulation compared with cells taken from uninfected controls. The most consistent depression occurred on day 28 post-infection.

c) The response of immune cells taken from donors infected with *Strongyloides spp.* to antigenic stimulation , although not consistent, seemed to follow the course of infection. On day 8 there was no increased blastogenesis initiated by specific stimulation, peak stimulation occurred on days 12-16, and by day 28, at the end of infection, no specific response was obtained.

d) Wherever antigenic stimulation occurred there was no consistent difference in the degree of stimulation obtained after homologous or heterologous challenge. No stage-specificity was expressed by the cellular response, nor did the nature of the antigen presented, that is, whether excretory/secretory or somatic, affect the response obtained.

e) One of the major consistent features of all experiments carried out was a high between rat variance in the response of cells from individuals to the same treatment. This occurred even with cells from uninfected controls.

f) The system could not detect a fourfold difference in numbers of cells responding to the mitogen, concanavalin A, at the range of concentrations used.

# CHAPTER 10

# DISCUSSION

The aim of this study was to investigate the inter-specific and intra-specific cross-immunity between three parasites within the genus *Strongyloides*. The essential criteria recorded to measure the effect of the host's immune response in this context were:

a) The proportion of the dose recovered post-infection. In the majority of cases rats were infected with an "exact" dose of $<100$ third-stage larvae of the relevant species/strain.

b) The distribution of adults along the intestine.

c) The number of eggs *in utero* per worm.

Change in the proportion of the dose recovered was a clear cut measure of the host's anti-worm response but the most sensitive criterion proved to be changes in the egg data. In some cases a reduction in the number of eggs per worm was obtained either before, or without, an effect on the number of worms. For example, in Expt 7, Chapter 7 (page 126) a reduction in terms of eggs *in utero* was obtained in the group given a homologous challenge of adult homogonic *S.ratti* worms on day 4 post-challenge, whereas a corresponding reduction in worms was not present until day 8. Similarly, in the adoptive transfer experiments, a reduction in egg numbers was present on day 8 post-challenge but a reduction in worms was not obtained until day 16 (page 151).

An attempt was made to discover whether the reduction in the number of

eggs *in utero* per worm of challenged rats compared with controls was, indeed, a true reflection of reduced fecundity. This was done by measuring the number of third-stage larvae produced per gm of faeces per worm in one of the experiments (page 109). It was possible that worms could lay eggs at a faster rate in immune animals with the result that they spent a shorter time in the uteri. At any one instant, therefore eggs *in utero* would be fewer in number, even though there was no change in egg production (i.e. fecundity). Rats from both homologous and heterologous challenge treatments did produce fewer infective larvae per gm of faeces per adult parasite compared with their respective controls, but there was a high variability within each treatment. Comparison of the extent to which eggs *in utero* and third-stage larvae in faeces were fewer in challenged animals compared with controls showed no precise correspondence, though the data are too scanty to draw firm conclusions (see ratios $R_E$ and $R_{L3}$, page 109). In practice, the number of third-stage larvae produced per gm of faeces was probably not the best measure of worm fecundity since there is a high variability in larval production under apparently identical culture conditions (page 72 ). Had it been possible, it would have been better to measure the actual egg production in the faeces of rats, but, in the case of *S.ratti*, a proportion of the eggs hatches in the intestine, so the numbers of first-stage larvae produced would also have had to be counted. Many workers do relate a reduction in the number of eggs *in utero* per worm with a reduction in worm fecundity with no supporting evidence. Moqbel and Wakelin (1979), Moqbel and McLaren (1980), and Moqbel *et al.* (1980), all refer to a reduction in this parameter as a reduction in the fecundity of *S.ratti*, but they do not include results to show that there has been a corresponding reduction in the number of eggs or larvae produced per worm to confirm the statement. Therefore, lacking firm evidence on the matter

from this study, a change in the number of eggs *in utero* per worm will be referred to without any implication that it is directly related to worm fecundity. This constraint in no way diminishes the value of the parameter as a measure of the host's anti-worm reaction.

Throughout this study, the location of adults within the intestine was noted, since some workers have found that a longitudinal migration within the intestine was associated with a developing host immune response which resulted in worm expulsion. An anterior migration has been found for *N.brasiliensis* (Brambell, 1965; Alphey, 1970), and a posterior migration for *T.spiralis* (McCoy, 1931; Kennedy, 1980), and *S.ratti* (Moqbel and Denham, 1977; Moqbel and McLaren, 1980; Moqbel and Wakelin, 1981). There seemed to be little evidence for a similar posterior migration of *S.ratti* or *S.venezuelensis* from my results. In a few cases there may have been posterior movement of *S.ratti* worms, but the majority were normally recovered from the first section of the intestine; for example, worms recovered from challenged treatments in Expts 2, 3, 5 and 7 (Chapter 5), seemed to be located more distally in the intestine compared with corresponding controls (page 313 ). However, in immune animals this observation was based on the relative position of only a few adults (generally less than 10), therefore it may have no general applicability. The apparent contradiction between present results and those of Moqbel and co-workers may be related to the size of dose, since an infection dose twenty-times smaller than that of those researchers was used. The fewer adults present in the experiments reported in this study may have sensitized a comparatively small area of local foci in the anterior intestine, and were thus able to move to a new site within the same region to escape any detrimental effects inflicted by the host's immune response. If there were many worms present, a large area of the anterior intestine may become sensitized and adult

parasites may have to move posteriorly to find a more hospitable site.

There seemed to be a difference in the distribution of *S.ratti* and *S.venezuelensis* in Edinburgh colony Wistar rats, but the two species did overlap. Adult *S.venezuelensis* parasites were apparently restricted to the first three sections of the intestine whereas *S.ratti* worms were recovered as far as section five (e.g. controls of Expts. 8 and 9, Chapter 5, page 316). This may be related to the small number of adult *S.venezuelensis* found post-infection, but 85 worms recovered from primary controls in Expt 11, Chapter 7, still exhibited a similar distribution (page 329). It would not be surprising if the two species had a different distribution since if they are to co-exist they must not exhibit total niche overlap (Schad, 1963). The fact that the longitudinal distribution of the two species overlaps to some extent does not mean they would be competing directly in that zone. Wertheim (1970b) suggested that the two species have a different radial distribution, and in the experience of this researcher, *S.venezuelensis* parasites are located nearer to the gut lumen than *S.ratti*.

### 10.1. Preliminary studies

One of the most vital of the preliminary experiments was to ensure that skin-penetrating third-stage *Strongyloides spp.* which could have developed from the faeces of rats carrying a worm burden, did not re-infect the animals. If this had occurred it would have complicated all the long term experiments. It was reassuring therefore, that, even when rats were not regularly cleaned out, the in-contact control animals did not become infected (page 60). Nevertheless, throughout this study the cleaning regime tested in the alternative treatment in this experiment was adopted, to ensure that there was

no possibility of rats re-infecting themselves.

It has been suggested by Moqbel and Denham (1978) that auto-infection occurs in *S.ratti*, whereby first-stage larvae which have hatched in the host's intestine could develop directly to the adult in an immunosuppressed host. There was no reason to suggest that rats in the experiments reported here were immunosuppressed, but it was necessary to devise some test to see whether auto-infection was a common event, because it could have had profound effects on the results. Therefore the surgical transfer technique was used to introduce first-stage *S.ratti* larvae directing into the host's intestine (page 64). As no worms were recovered from any of the rats thus infected, it was assumed that auto-infection, if it occurs at all, is a rare phenomenon which can be safely excluded as a complicating factor in the interpretation of experimental results.

Graham (1939) thought that there was a seasonal variation in the production of free-living adults from faecal cultures of his heterogonic strain of *S.ratti*, which reflected subliminal changes in the environmental conditions to which the host was exposed. If there was a similar seasonal variation in the number of third-stage larvae produced in cultures, it could reflect changes in the egg production of parasitic females in the gut. This would have meant that measurements of the number of eggs *in utero* per worm, one of the criteria used to record an anti-worm response, could have been suspect. Therefore the output of infective larvae from faecal cultures of the three types of parasite used in this study over the course of two years was studied. Fortunately, no regular pattern of the sort in question emerged (page 72). In this experimenter's experience variations in the number produced were due to random changes in the uncontrolled conditions of faecal culture. Few larvae

were collected if the paper towel on which the faecal pellets were collected was either too wet or too dry: if faecal cultures were made too wet at the beginning, or during incubation, very few larvae emerged. These parameters were a matter of personal judgement and only a very detailed study would identify the optimum conditions to ensure that the majority of larvae from a given faecal sample was obtained. Even if such optima could be defined, it would probably be impossible to conform to them under routine culture conditions.

Most of the experiments in this study ran for 5-6 weeks and, in order to accommodate all the animals necessary, rats had to be housed together. Bailenger and Cabannes (1976) have shown that changes in the blood corticosteroid levels can have an antagonistic effect on the non-specific inflammatory response, leading to a higher *S.ratti* worm burden. Stressful situations are among the factors which cause alterations in the hormone levels; so it had to be established that a method of housing rats which might create stress due to an increase in the social interactions between animals, would not result in higher worm burdens. The results of experiment 1, Chapter 3 (page 58) provide firm evidence that this possibility could also be disregarded in the interpretation of results.

A major problem encountered in this study was a resistance to infection with *S.venezuelensis* in Edinburgh colony Wistar rats. A potential way of overcoming this was to introduce adult *S.venezuelensis* worms directly into the intestine of the host, since it was possible that the innate resistance was expressed during the parasite's migration and development. Before comprehensive, comparative studies of surgical transfers using adult worms of all three types of parasite to prime rats were attempted, a number of

preliminary experiments were undertaken to determine the optimum technique to apply. It was found that worms had to be transferred within two hours of the donor's death, since the time adults spent in the extra-intestinal environment could have a detrimental effect on their survival post-transfer (page 62). Kennedy (1980) found a similarly depressed worm survival if adult *T.spiralis* were transferred later than two hours after the donor's death. To avoid such damage, the deaths of the rats which supplied the worms to be transferred were sequential, since one rat could only supply enough adults to infect 4-6 rats with an exact dose of <50 worms (page 61). In order for this to be valid experimentally, it was necessary to show that worms from different donors were not significantly different in terms of viability. It was reassuring therefore to find that adults aquired from different hosts had equal capabilities of establishing in a new rat (page 63).

Another method used to carry out reciprocal challenge experiments with both strains of *S.ratti* and *S.venezuelensis* was adoptive transfer of mesenteric lymph node cells between rats of the PVG inbred line (page 149). Initially it had to be ensured that injection of the medium in which the cells were suspended had no effect on the worms which might have been interpreted as immunological. It was found that the medium had no such effects, therefore any changes following the transfer of immune cells could be attributed to the influence of the cells themselves (page 66).

In preliminary studies of the lymphocyte transformation assay, the effect of three factors on the level of stimulation obtained was investigated as follows: serum concentration in the cell medium, mitogen concentration, and the day of harvesting the cells. Human serum at a concentration of 15% was used to supplement the cell medium since this produced the highest level of

mitogen-induced stimulation of the three concentrations tested (page 67). Paradoxically, this also caused the maximum suppression of unstimulated cell activity, implying that it may be blocking some response at this concentration; but since the most important aspect in these experiments was the amount of specific stimulation produced, this was disregarded. A high level of stimulation was produced using concanavalin A at a concentration of 2.5µg/ml so it was decided that this concentration would be used throughout experiments (page 68). The results of Expts 11 and 12 showed that peak mitogenic response was demonstrated if cells were harvested on day 3 of culture (page 70). However, the response to antigenic stimulation may not occur as early as this. Genta *et al.* (1983) did not harvest rat cells stimulated with an *S.ratti* antigen until at least 79 hours post-stimulation, and Bloch *et al.* (1977) harvested theirs, treated with a *N.brasiliensis* antigen, after 90-96 hours of culture. Taking all things into consideration, it was decided that cells would be harvested on day 4 of culture, when a good response to mitogenic stimulation was still obtained, and cells would have been exposed to the antigen for a prolonged period.

The preliminary experiments on lymphocyte transformation were carried out using cells from uninfected rats. Ideally they should have been repeated with cells from individuals infected with *Strongyloides spp.* to ensure that conditions were equally suitable in this context. Due to financial restrictions this was not possible. The experimental protocol used was based on that of Genta *et al.* (1983), who investigated the effect of stimulation of mesenteric lymph node cells with a *S.ratti* antigen, therefore it seemed unlikely that the conditions would be unfavourable. In conformity with this protocol, a concentration of $2 \times 10^5$ cells per well was adopted as standard since, with it, a good level of mitogen-induced stimulation had been obtained (but see later).

Before reciprocal challenge experiments using full infections of all three *Strongyloides spp.* parasites to prime animals could be carried out, it was necessary to discover how long a primary infection with an exact dose of <100 infective larvae would last. It was essential to know this in order to challenge rats when the effects of the host's inflammatory response against the priming infection had subsided. Unfortunately it was only possible to investigate this aspect for the two *S.ratti* parasites, because infection of Edinburgh colony Wistars rats with a similar small dose of *S.venezuelensis* led to an insignificant adult worm burden (page 84). Results showed that the majority of adult worms from a primary infection of an exact dose of less than 100 third-stage *S.ratti* had been expelled by day 28 post-infection (page 84). Previous workers, using infection doses 10-40 times larger, found that the duration of a primary infection of *S.ratti* had a similar time span (Moqbel and Denham, 1977; Moqbel and Wakelin, 1981).

There seemed to be a difference in the pattern of worm loss exhibited by the two strains. Two replicate experiments (Expts 1 and 2), using the homogonic strain revealed similar rates of loss. This was in spite of a significant difference in the proportion of the dose obtained at each sampling time. In the single relevant experiment (Expt 3), the heterogonic strain suffered progressively greater losses than the homogonic strain, illustrated by the higher exponential coefficient in that case (page 91). Whether this was due to a more severe specific anti-worm response against the heterogonic strain, or to a non-specific innate resistance, could be debated. The former would be consistent with results from some of the adoptive transfer experiments carried out, since no dose-response effect was exhibited in homologous adoptive resistance of rats primed with immune cells obtained from donors infected with two widely different levels of the heterogonic strain.

From this it was apparent that <100 larvae produced just as potent an immune response in cell donors as infection with 2000 (page 157). In contrast, a dose-response effect was expressed if the homogonic strain of *S.ratti* was used in the same protocol, demonstrating that an exact dose of <100 larvae produced a less severe immune response in cell donors (page 157). Thus the heterogonic strain of *S.ratti* might be a more potent stimulator of the host's specific immune response. On the other hand, the host may express a higher level of innate resistance against the heterogonic strain, since a consistently lower worm burden was obtained for this parasite, compared with the homogonic strain, in naive Wistar and PVG strain animals (Table 10:1, page 227). Therefore a more severe innate response against the parasite, coupled with specific events, may explain the phenomenon. Further experimentation would be necessary to decide between these alternatives.

There was also a reduction in the number of eggs *in utero* per worm over the course of infection with *S.ratti* but, as no measurement of the egg or third-stage larval production was taken, it was not known if this reflected a reduction in worm fecundity. The data of Moqbel and Denham (1977) can be used to show that worm fecundity did decrease over the course of their infections, since one can calculate that the number of third-stages produced per gm of faeces per adult worm fell from 12.7 on day 10 to 3.4 on day 32 post-infection. Unfortunately they did not refer to this specifically or measure the number of eggs *in utero* per worm. Had they done so, it could have settled the question of the relationship of the two parameters. There seemed to be an equilibrium level of two eggs *in utero* per worm in the present experiments, reached on day 26 for the homogonic strain, and on day 20 post-infection for the heterogonic. This contrasts with the results of Moqbel and McLaren (1980) and Moqbel and Wakelin (1981), who recorded a value of

Table 10:1

Egg and worm data collected throughout this study

| Parasite | | proportion of the dose recoverered S/A | I | S/A day 16 | mean number of eggs in utero per worm | per worm per rat |
|---|---|---|---|---|---|---|
| S.venezuelenis | $\bar{X}$ | 0.045 | | | | |
| (Wistar rats) | n | 6 | | | | |
| | SD | 0.04 | | | | |
| | $\bar{X}$ | | 0.047 | | | |
| | n | | 6 | | | |
| | SD | | 0.03 | | | |
| (PVG rats) | $\bar{X}$ | 0.353 | | | 6.841 | 6.821 |
| | n | 3 | | | 2 | 2 |
| | SD | 0.08 | | | 0.01 | 0.01 |
| | $\bar{X}$ | | | 0.296 | 6.522 | 6.500 |
| | n | | | 2 | 2 | 2 |
| | SD | | | 0.03 | 0.46 | 0.29 |
| homogonic | $\bar{X}$ | 0.562 | | | 7.224 | 7.246 |
| S.ratti | n | 25 | | | 22 | 15 |
| (Wistar rats) | SD | 0.08 | | | 0.39 | 0.42 |
| | $\bar{X}$ | | 0.501 | | 7.210 | 7.178 |
| | n | | 15 | | 13 | 13 |
| | SD | | 0.12 | | 0.84 | 0.83 |
| (PVG rats) | $\bar{X}$ | | | 0.423 | 6.883 | 6.859 |
| | n | | | 8 | 8 | 8 |
| | SD | | | 0.10 | 0.36 | 0.39 |
| heterogonic | $\bar{X}$ | 0.482 | | | 5.982 | 5.898 |
| S.ratti | n | 10 | | | 9 | 9 |
| (Wistar rats) | SD | 0.11 | | | 0.55 | 0.54 |
| | $\bar{X}$ | | 0.473 | | 5.967 | 5.744 |
| | n | | 6 | | 6 | 6 |
| | SD | | 0.07 | | 0.03 | 0.39 |
| (PVG rats) | $\bar{X}$ | | | 0.307 | 5.781 | 5.732 |
| | n | | | 8 | 8 | 8 |
| | SD | | | 0.04 | 0.77 | 0.73 |

S/A : skin-application

I : injection

0.0, and less that 0.5, eggs *in utero* per worm, respectively, on day 26 post-infection with *S.ratti*. This difference could be related to the use of different sized doses to infect rats, perhaps implying that a dose-dependent anti-worm response exists which could be demonstrated as changes in the egg data. Beyond the likelihood that they are immunologically mediated (see later), the actual mechanism or mechanisms responsible for the reduction in the egg and worm numbers in these experiments are unknown, but it is improbable that both are manifestations of the same isolated process for reasons to be discussed (page 243, *et seq.*).

### 10.2. Studies on *S.venezuelensis*

A number of experiments were carried out in an attempt to explain why only a few adults were recovered from Edinburgh colony Wistar rats infected with an exact dose of <100 third-stage *S.venezuelensis* larvae. Two of the factors investigated were found to have an effect; the age of the host and the size of the infection dose.

Increasing the size of the dose to 2000 third-stage *S.venezuelensis* seemed to increase the proportion recovered as adults, but it must be noted that no controls infected with small exact doses were included in the experimental protocol (page 114.). This was the reverse of what was found by Wertheim (1970b), for, in her experiments, a dose of 2000 led to an 82% reduction in the proportion recovered compared with one of 250. The only difference between this study and that of Wertheim would appear to be the strain of rat involved.

There was a definite age-related resistance to infection demonstrated in Edinburgh colony Wistar rats, since a significantly greater proportion of the dose was recovered from three-week old, compared to adult, rats at 72 hours

using labelled larvae, (page 115), and on day 8 post-infection, when worms were counted directly (page 114). However, the proportion recovered from three-week old rats was still significantly lower than that obtained from similarly aged animals infected with *S.ratti* (page 115).

The basis of the age-related resistance is unknown, but it could be due to a number of factors: either physical, physiological or associated with fundamental differences in the ecology of this parasite compared with *S.ratti* (see later).

Lewert and Lee (1957) found that susceptibility of the host to infection with *S.ratti* decreased with age and that, at the same time, the ability of infective larvae to penetrate the skin was reduced. The physiology of the host can have an effect since the immune response of rats against *N.brasiliensis* was shown not to be fully developed until they were 7-9 weeks old (Ogilvie and Love, 1974), implying that some component involved in the expulsion of the parasite is not fully developed until the rats reach a certain age. This does not seem to be the case for *S.venezuelensis*, since 3 week old rats, primed with an exact dose of <200 infective *S.venezuelensis* larvae, express a significant immune response against heterologous challenge with both strains of *S.ratti* given 9 weeks later (page 141).

A similar age-related resistance was demonstrated for *S.ratti* by Zamirdin (1974) who worked in this laboratory. He obtained a significantly higher proportion of an exact dose of less than 100 infective *S.ratti* (homogonic strain) from three-week old rats compared with that obtained from 9 or 12 week olds. However, the same effect was not demonstrated with this particular parasite in this study (page 115).

It was not fully appreciated until experiments using PVG rats were started what a profound effect the strain of the host could have in this context. Higher adult *S.venezuelensis* recoveries were obtained from this strain of rat compared to that obtained in Wistars (Table 10:1, page 227). For example, a proportion of 0.421 of an exact dose of <100 larvae was recovered on day 8 post-infection from PVG animals (page 352); whereas a proportion of only 0.013 was obtained from Wistars (page 321), infected with a similar small dose. This may explain why these results contrast with those of other workers. Wertheim (1970b) recovered 44% of a dose of 250 infective larvae in "Sabra" strain rats, and Nolan and Katz (1981) recovered 3.16% of an inoculum of 4000 third-stage larvae from "Sprague-Dawley" animals, both on day 7 post-infection.

The experiments carried out using radio-labelled larvae led to some important conclusions. First, the results showed that *S.venezuelensis* infective larvae lost a third of their attached label during their development (page 115). Seaton (personal communication) has found that 50% of the same radio-active label attached to *Ostertagia circumcincta* third-stage larvae was lost during exsheathment. Thirty-seven percent could be accounted for on the sheath, the remaining 13% was probably metabolised away. Similarly, Georgi and Le Jambre (1983), who also used $^{75}Se$, found that 40% of label attached to infective *Haemonchus contortus* larvae was lost during chemically-induced ecdysis, 17-32% being lost in a soluble form. Therefore, in order to carry out critical studies using this method, it would be useful to obtain some sort of correction factor for all three *Strongyloides spp.* which would take into account the amount of label lost during their development.

Assuming that the homogonic strain of *S.ratti* and *S.venezuelensis* lose a

similar proportion of their attached radio-label, the results of Expt 5 (page 115) would indicate that the migration of *S.venezuelensis* took longer, since third-stage *S.venezuelensis* larvae were apparently still reaching the intestine after 72 hours post-infection. Seaton (1983), who worked in this laboratory with $^{75}Se$ labelled larvae, found that the migration of *S.ratti* was completed after 40 hours, but results of other workers using traditional methods have implied that it has not terminated until after 96 hours post-infection (Abadie, 1963; Wertheim and Lengy, 1965; Tada *et al*, 1979). The report of Wertheim (1970a) is at least in agreement with the data collected in this study in so far as the migration of *S.venezuelensis* took longer that that of *S.ratti*. Wilson *et al.* (1986) have discussed the reasons why traditional methods may lead to an over-estimate of such periods of migration.

Piecing together various bits of information collected about the biology of *S.venezuelensis* in this study, one can put forward some reasons for the low worm burden obtained in Edinburgh colony Wistar rats. First, a higher proportion of the dose was recovered from three-week old rats compared with their adult counterparts (pages 114, 115 and 141). Second, a significantly higher proportion of the dose was recovered from 3 week old rats compared with adults at three days post-infection, implying that in adult rats infective larvae were not reaching the intestine (page 115). Third, a cumulative *S.venezuelensis* worm burden was not obtained in rats exposed to a trickle infection (page 134).

One way to interpret these results would be to assume that the transmammary route of infection, which has been demonstrated in a number of *Strongyloides spp*; for example, *S.ratti* in rats (Zamirdin and Wilson, 1974; Wilson *et al*, 1976a, 1976b, 1978, 1981, 1982), *S.westeri* in equines (Lyons *et*

*al.* 1973, 1977), and *S.ransomi* (Moncol and Batte, 1966; Stewart *et al.* 1969, 1976); predominates in *S.venezuelensis*. Let us assume that the majority of third-stage *S.venezuelensis* larvae which infect adult rats form a larval tissue pool available for milk-borne infection. Therefore, if the rat is female and becomes pregnant, physiological triggers, induced by hormonal changes in the host, activate the larvae to migrate to the mammary gland for passage into the pups. Once transferred to a susceptible host all the larvae pass to the intestine and develop into parasitic female worms. These produce eggs which pass into the external enviroment and develop into the skin-penetrating infective larval stage and so the cycle begins again. It would be relatively simple to discover if this theory applies since treatment of rats with corticosteroids should be able to demonstrate if infective larvae of *S.venezuelensis* do undergo an arrested development in the adult host. So far, apart from the fact that milk-borne infection does occur (Nolan and Katz, 1981), insufficient is known about it in *S.venezuelensis*. Comparative studies in this laboratory of transmammary infection by *S.ratti* leads to the conclusion that hypobiosis is not involved in that case (Wilson, personal communication). There is an anomaly in that *S.venezuelensis* develops well in adults of the "Sabra" (Wertheim, 1971) and PVG (Table 10:1, page 227) strains of rat. However, it is common to find specificities according to the strain of host associated with unnatural host/parasite combinations, and, possibly, the adult rat is equivalent to an unnatural host in this case.

### 10.3. Framework for the study of cross-immunity between *S.ratti* and *S.venezuelensis*

From the preliminary experiments it emerged that it was impossible to prime adult Edinburgh colony Wistar rats against *S.venezuelensis* using a full

infection, so that other methods to carry out reciprocal cross-challenge experiments *in vivo* were investigated, in the hope that one or more would provide a suitable experimental system. Four methods were tested and of these, priming with adult worms, and full infections in immature rats, showed some promise of success.

No protective response was obtained in homologously challenged rats primed with a single dose of 10,000 heat-killed third-stage homogonic strain *S.ratti* larvae (page 122). As it had already been found that *S.ratti* was a potent stimulator of the host's immune response following the normal sequence of infection (page 93), it seemed pointless to continue further studies using this protocol. Recent experiments by Conder and Williams (1983), where rats were also immunised with a single injection of dead infective *S.ratti* larvae, suggest that the immune response thus initiated does not affect the ability of worms to establish, but that their egg production and the mean number of eggs *in utero* per worm are reduced. Unfortunately, this parameter was not measured in this experiment. It might be interesting to carry out further studies, using the present protocol, or that of Conder and Williams, to discover if strain and / or species specificity is reflected in differences in eggs *in utero* in response to priming with dead larvae.

Exposing rats to a trickle infection of *S.venezuelensis* for 6 or 10 weeks did not immunise animals against subsequent challenge infection with an exact dose of less than 100 infective homogonic *S.ratti* larvae, nor did it lead to cumulative *S.venezuelensis* worm burdens over the course of the experiments (page 132). In contrast, Jenkins and Phillipson (1970) were able to protect rats, primed using a trickle infection of *N.brasiliensis*, against homologous challenge; and they also obtained a cumulative worm burden. However, they

used very young rats in their experiments, which have been shown to have a reduced ability to expel adult *N.brasiliensis* worms (Jarrett *et al*, 1966, 1968), and it has also been shown that the rat's immune response against *N.brasiliensis* does not become fully competent until animals reach 7-9 weeks of age (Ogilvie and Love, 1974); therefore the difference could be due to the use of different aged rats in the two studies.

Priming rats with surgically transferred worms seemed to offer a method for investigating the antigenic relationship of the three *Strongyloides spp*, but, unfortunately, the high variability obtained within treatments reduced the sensitivity of the assay, so that only gross changes in the host's immune response would be identified (page 126). Therefore, this method of comparison was abandoned since it was very unlikely that the assay would have detected small differences in the host's immune response against the parasites. The egg data seemed less affected by this overall variability because, in both of the relevant experiments (see above), there was a significant reduction in the number of eggs *in utero* per worm in challenged treatments compared to their respective controls. The host was also expressing some specificity in Expt 8 since homologous challenge produced a significantly greater relative reduction in uterine eggs compared with its control (page 135). It may be rewarding to carry out comparative studies which concentrate wholly on this measurement of the host's anti-worm response, but, unfortunately, it was impossible to embark on a new framework of experimentation midway through this project.

The most promising results came from immature rats primed against a full infection of *S.venezuelensis*. Three-week old animals, given an exact dose of <200 third-stage *S.venezuelensis* larvae, produced a significant resistance

against homologous and heterologous *S.ratti* challenge 9 weeks later, which appeared as a reduction in the number of eggs *in utero* per worm for all treatments as well as a reduction in the proportion of the dose recovered after heterologous challenge, compared with the respective controls (page 141).

Taking the analysis further, quantitative comparison of the immunity induced using the worm data revealed no significant difference in the degree of protection obtained after challenge when all three parasites were considered. This outcome was not surprising because there was a high variability within the data for homologous challenge (coefficient of variation for this was 127%, whereas it was only 21% for homogonic *S.ratti* challenge, and 27% for heterogonic *S.ratti*). If the homologous challenge group was omitted from the analysis, it became apparent that heterologous challenge with the heterogonic strain of *S.ratti* produced a significantly greater level of immunity in the rats (page 145). The data for eggs *in utero* from this experiment were more homogeneous. Comparison of the level of resistance produced by all three treatments, on this basis, demonstrated the existence of a graded immune response against the parasites (page 145). Homologous challenge elicited the maximum level of immunity, followed by heterologous challenge with heterogonic *S.ratti*, and the minimum amount was produced by homogonic *S.ratti* challenge . This confirms the results of the worm data and it also implies that there is some antigenic diversity between the two *S.ratti* strains (see later)

The high variability within the homologous challenge treatment implied that "responder" and "non-responder" rats may exist within the rat population, which vary in their ability to expel *S.venezuelensis*. Three of the seven rats within the homologous treatment expelled all their worms, whilst adults were

recovered from the remaining four animals. The existence of "responders" and "non-responders" within the host population has been identified for other parasites: *T.muris* in mice; Wakelin, 1975; *T.spiralis* in mice; Bell *et al*, 1982a and 1982b; (also see review by Wakelin, 1978).

The previously described age-related resistance to infection of Wistar rats with *S.venezuelensis* was also demonstrated in this experiment, since a significantly higher proportion of the dose was recovered from primary compared with secondary controls (page 143). Adult *S.venezuelensis* parasites were recovered from the heterologous challenge treatments, and these worms must have been exposed to an immune response since rats harbouring this infection, when challenged, were resistant to re-infection with *S.ratti*. Therefore, the adult *S.venezuelensis* had somehow managed to survive despite being in a hostile environment. There was no significant difference in the proportion of the dose recovered of this species from any of the challenged groups, or the secondary controls. It is posible, therefore, that there is a constant proportion of *S.venezuelensis* worms within the population which is specially adapted to mature in older hosts and to evade the rejection mechanism of the immune response.

Further studies with young rats were not pursued, because information on the amount of cross-immunity between the two strains of *S.ratti* had already been collected using adult hosts. The results of reciprocal challenge experiments with *S.ratti* primed animals would have been difficult to interpret, because of the age-related resistance to *S.venezuelensis*.

The antigenic relationship of the three parasites was also studied using an *in vitro* assay of immune lymphocytes, with variable results. Nevertheless, some very interesting results did come out of these experiments which, for

simplicity will be discussed in the order of the three main types of treatment used; i.e. the behaviour of unstimulated controls, the response of cells exposed to concanavalin A, and their response after treatment with antigens.

There seemed to be a higher level of unstimulated activity in immune cells, compared to their normal counterparts, which followed the course of infection (Table 10:2, page 239). Although technically "non-specific", the increased activity could indicate that the cellular response against *Strongyloides spp.* did not begin until after day 8, that it peaked on days 12-16, and then dropped back to control levels by day 21 post-infection. This increase in non-specific blastogenesis may be a general feature of parasitic infection, since it has been reported in other systems; for example, in *Trypanosoma brucei* in mice (Corsini *et al*, 1977); *Plasmodium falciparum* in mice (Greenwood and Vick, 1975); *Plasmodium berghei* in mice (Golsener *et al*, 1975); *Toxoplasma gondii* in mice (Strickland *et al*, 1975), and *N.brasiliensis* in rats (Bloch *et al*, 1977); and it is often related to the course of the infection. The effect may be restricted to cells present in the lymphatic organ draining the site occupied by the worms, since Bloch *et al.* (1977, see above) only obtained an increased activity in mesenteric lymph node cells: spleen cells from the same individuals did not exhibit the phenomenon.

The mitotic activity initiated *in vivo* was still high after 80 hours of culture *in vitro* in the present experiments, so that it was likely that cells went through more than one cycle of DNA replication. A similar observation probably applies to cells obtained from rats infected with *N.brasiliensis* in experiments by Bloch *et al*, since they still incorporated label between 73-96 hours after the start of *in vitro* culture. However, in rats infected with *P.berghei* increased unstimulated activity of spleen cells was restricted to the

first 8 hours of culture. Possibly in that case, therefore, *in vivo* stimulation initiated just one cycle of DNA multiplication (Golsener *et al*, 1975). It would be interesting to characterise the increased unstimulated response in immune cells from rats infected with *Strongyloides spp*, to discover how long it could be sustained *in vitro*, when the peak activity occurred, what the products of the cells were, and whether there was any strain or species-specificity demonstrated by the response.

The activity of cells treated with concanavalin A was intended as a positive control to check that they were capable of responding to stimulation, but these results also yielded some information on the events which may have been occurring *in vivo*. On day 8 post-infection there was no difference in the response of immune and normal cells to mitogenic stimulation; on days 12 and 16 an apparently depressed response was sometimes obtained, but on days 21 and 28 post-infection there was a definite reduced response by immune cells (Table 10:3, page 240). When the depressed response was obtained on days 12 and 16, it was generally an artifact inherent in the method of analysing the data, since a relatively higher level of unstimulated activity present in immune compared with normal cells at this time (see earlier) meant that a lower differential count was obtained for them. The actual number of counts per minute obtained for the two cell populations in response to mitogenic stimulation was similar. Thus cells from infected individuals were probably expressing a near maximal response to stimulation before they were placed in culture.

A depression in the response to concanavalin A stimulation was elicited in immune cells taken from rats infected with *Strongyloides spp.* on day 12 post-infection in Expt 4, and on day 28 in Expts 1 and 2

Table 10:2

Unstimulated blastogenesis: immune vs normal mesenteric lymph node cells

| Parasite used to prime cell donors | Day post-infection | Expt | Activity compared with normal cells elevated | depressed | no change |
|---|---|---|---|---|---|
| heterogonic S.ratti | 8 | 1 | ✡ | | |
| S.venezuelensis | | 3 | | | ✡ |
| S.venezuelensis | 12 | 3 | | | ✡ |
| | | 6 | ✡ | | |
| heterogonic S.ratti | | 5 | ✡ | | |
| | | 7 | | | ✡? |
| | | 8 | ✡ | | |
| homogonic S.ratti | | 4 | | | ✡? |
| heterogonic S.ratti | 16 | 1 | | | ✡ |
| | | 7 | | | ✡ |
| | | 8 | | | ✡? |
| S.venezuelensis | 21 | 3 | | | ✡ |
| heterogonic S.ratti | 28 | 1 | | | ✡? |
| | | 2 | | | ✡? |

Table 10:3
Concanavalin A stimulation: normal vs immune cells

| Parasite used to prime cell donors | Day post-Infection | Expt | Activity relative to normal cells: elevated | depressed | no change |
|---|---|---|---|---|---|
| heterogonic *S.ratti* | 8 | 1 | | | ✡ |
| heterogonic *S.ratti* | 12 | 5 | | | ✡ |
| | | 7 | | | ✡? |
| | | 8 | | | ✡ |
| | | 6 | | | ✡ |
| homogonic *S.ratti* | | 4 | | ✡ | |
| heterogonic *S.ratti* | 16 | 1 | | | ✡ |
| | | 7 | | | ✡? |
| | | 8 | | | ✡ |
| *S.venezuelensis* | 21 | 3 | | | ✡ |
| heterogonic *S.ratti* | 28 | 1 | | ✡ | |
| | | 2 | | ✡ | |

(chapter 9). In Expt 4 a specific response occurred to antigenic stimulation whereas in Expts 1 and 2 there was no response (Table 10:4, page 242), suggesting that cells with specificities for *Strongyloides spp.* antigens were "switched off" during the later stages of infection, when a fully effective immune response had been completed. It would seem logical for the host to dampen its reaction against the parasites at a time when all the worms had been expelled in Expts 1 and 2, but a high number of adults was recovered from rats which supplied the immune cells for Expts 3 and 4. A similar depressed response to the mitogen phytohaemaglutinnin was not obtained by Dobson and Soulsby (1974), who studied the response of peripheral blood leukocytes from guinea pigs infected with the sheep parasite *Trichostrongylus colubriformis*. This could be due to the use of different cell populations in the two studies, since it has already been shown that an increase in the unstimulated non-specific activity of immune cells can be restricted to the site occupied by the worms (see earlier).

One of the problems associated with analysing the lymphocyte transformation data is differences in the unstimulated non-specific activity of different cell populations. A method commonly used in the belief that it overcomes this difficulty is to calculate the stimulation index for different treatments (i.e. "experimental" divided by "unstimulated control"). Unfortunately, as found in this study, this can lead to misleading results. For example, if the response of immune and normal cells to concanavalin A stimulation is considered then, if the unstimulated activity of immune cells is quantitatively much higher than that obtained for their normal counterparts, it would lead to a much lower stimulation index for treatment with the mitogen even though the actual number of counts per minute for the two cell populations may be very similar. The apparent depression in response is an

Table 10:4

Cells from uninfected and infected animals were exposed to various antigenic preparations. A significantly greater response in the cells from infected rats was taken as an indication of a specific response to the antigen. This table summarises the data collected from my studies and shows which antigens produced a positive response in a particular test. Thus the effect of homologous and heterologous challenge of the cells can be compared.

Table 10:4
Specific response to antigenic stimulation

| Parasite used to prime cell donors | Day post-infection | Expt | Antigen added to cells: Adult E/S | | | Adult homogenate | | Larval | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | HO | HE | S | HE | S | HE | HO | S |
| heterogonic *S.ratti* | 8 | 1 | | | | - | | | | |
| *S.venezuelensis* | | 3 | | | - | | - | | | |
| heterogonic *S.ratti* | 12 | 5 | + | - | - | | | | | |
| | | 7 | + | - | - | | | + | + | + |
| | | 8 | + | + | + | | | + | - | |
| *S.venezuelensis* | | 3 | + | | | | + | | | |
| | | 6 | - | - | - | - | | | | |
| homogonic *S.ratti* | | 4 | - | + | + | | | | | |
| heterogonic *S.ratti* | 16 | 1 | | | | - | | | | |
| | | 7 | - | - | - | | | - | - | - |
| | | 8 | + | + | + | | | + | - | |
| *S.venezuelensis* | 21 | 3 | | | + | | + | | | |
| heterogonic *S.ratti* | 28 | 1 | | | | | | - | | |
| | | 2 | - | - | - | | | | | |

Where:-

HO = homogonic *S.ratti* antigen
HE = heterogonic *S.ratti* antigen
S = *S.venezuelensis* antigen
E/S = excretory/secretory antigen

artifact of the method of analysis. As already pointed out, the "differential count" method adopted in the present work, if viewed uncritically, could lead to false conclusions in some cases; but the parameters in the equation can be identified with greater clarity. Suffice it to say that no study of this sort should be reported without a full record of the unstimulated control values.

The "switching off" of cells at the end of infection may explain why a time lag occurred between administering immune cells, and the effects they brought about, in homologous challenged rats in the adoptive transfer experiments reported here (see later). Taking these experiments into consideration, if the worm data was used to assess resistance then the recipients of cells killed on day 16 post-challenge expressed a significant immunity whereas those killed on day 8 did not, even if the number of immune cells transferred was increased four-fold (page 151), or if the infection level in the donors was increased (page 151). However, if the transferred immunity was assessed as a reduction in the number of eggs *in utero* per worm compared with secondary controls, then a significant immunity was expressed on both days 8 and 16 post-challenge (page 151). Thus it could be thought that an anti-worm response had been initiated by day 8, but it could only be detected as changes in the number of eggs *in utero* per worm, which seems the more sensitive of the two measures of the host's anti-parasitic response used in this study.

Moqbel and Wakelin (1981) also found that a reduction in the *S.ratti* worm burden, after adoptive transfer of immune cells, did not occur until day 16 post-challenge. Moreover, they showed that if cells were given on day 6 post-infection worm loss still began on day 16, when cells had only been present for 10 days. It appeared from this that it was not the length of time

that the cells were present which was important, but the length of time the host was exposed to enteral stimulation. Moqbel and Wakelin (1981) also obtained a reduction in uterine eggs before worm expulsion, but, surprisingly, they found that it did not occur until day 14 post-challenge, even though their experimental protocol was similar to the one used in these experiments. The decrease they described was 6 days later than that obtained in the present study, even though the doses they used to prime cell donors and to challenge recipients were twenty times larger. This difference in results could not be due to the use of different strains of parasite because the stock homogonic variety was common to our laboratories (Wilson, personal communication), or to the use of different strains of rat, since PVG animals were used in both studies, although the source was not the same.

Moqbel and Wakelin (1981), suggested two theories to explain their results:-

i) the reduction in the worm burden and the reduction in the number of eggs *in utero* per worm was brought about by cells with the same specifities, but that their effects had different quantitative cellular requirements.

ii) the reduction in the two parameters was caused by qualitatively different mechanisms, and there was a relatively greater proportion of cells which caused changes in egg content compared with those which led to worm expulsion.

However, there is another way in which the results of Moqbel and Wakelin and those obtained in the present study could be interpreted. If one assumes that cells with the same specificities cause a spectrum of effects on several organ systems, which vary in susceptibility; then effects which are related to

different sensitivities, independent of cell numbers, could be temporally separated purely because the rates at which they are brought about are different. Which parameter is recorded is a subjective matter, and usually the most easily measured effect, such as changes in worm length or changes in the number of eggs *in utero* is chosen. Not surprisingly, these all occur before worm expulsion, which is the result of an accumulation of processes having a variety of underlying causes. Thus just because events are temporally separated does not mean that they are caused by different mechanisms, or by different numbers of cells with the same specificity.

Results from the studies of lymphocyte transformation show that such cells are suppressed by day 28 of an infection, presumably by the activity of the T-suppressor subset. Therefore they may not be able to respond to antigenic stimulation by the challenge infection until a primary immune response (starting about day 8 post-infection) had been elicited in the recipient. Mediators produced by responding T-helper cells from the recipient's cell population would be able to reverse the suppressive effect of the transferred T-suppressor cells so that the host now had available a population of primed lymphocytes with specificites for the parasite to aid in its immune response. Armed in this way, it would be able to mount a quicker and more severe anti- worm response than would have been possible had it not been immunised. This theory would explain why Moqbel and Wakelin found that cells given either on day 1 or on day 6 post-infection caused a reduction in worms at the same time, i.e. day 16. It would be interesting to investigate the effect on homologous challenge of transferring a mixed cell population of day 16 immune cells (which are actively responding to the parasite) and day 28 immune cells (containing an enriched population of memory cells with specificities for the parasite). Prediction from this theory is that an effect

would be present by day 8 post-challenge.

The results obtained after antigenic stimulation of cells *in vitro* express one main feature, that is, inconsistancy (Table 10:4, page 242). Wherever specific stimulation did occur there was no difference in the level obtained after homologous or heterologous challenge; no dose-response effect was demonstrated for any antigen preparation; nor did the nature of the antigen, that is, whether it was of somatic or excretory/secretory origin, seem to have an effect; and no stage-specificity emerged. The results were consistent with the idea that the cellular response against *Strongyloides spp.* did not begin until day 12, that it peaked on days 12-16, and had terminated by day 28 post-primary infection. Overall it appeared that, if antigenic stimulation occurred, then its intensity was similar for all antigenic preparations.

One of the major reasons for this result was probably because the antigens were used at too dilute a concentration. No protein seemed to be extracted from the parasites, either by homogenization or *in vitro* culture, even though the amount of raw material was increased and different techniques like freeze-thawing and sonication were tried. Perhaps the only method of ensuring that protein is stripped from the parasites is to use some detergent such as CTAB but, unfortunately, there was no time to start intensive studies to find the best detergent and the conditions for its application. Genta *et al.* (1983) were obviously able to extract some protein, and the only difference between their method and the one used in this study was that they used sodium hypochlorite to sterilise *S.ratti* third-stage larvae, which possibly could have made it easier to remove protein. It was found in the present study that there was a limit to the sensitivity of the Lowry assay used to standardise the protein concentration of the antigens, since it was only possible to detect

protein if it was above a concentration of 100ug/ml. Genta *et al.* also used this method to standardise their antigens. It would be interesting to see the sort of standard curves they obtained and the initial concentration of their antigens before dilution. There may also have been some fundamental defect in the experimental design used throughout the lymphocyte transformation described. Altering the cell density could improve the sensitivity of the assay, since the results of Expt 9 (page 211) gave cause to think that the one used ($2.5x10^6$/ml) was not suitable to detect small changes in antigen-induced proliferation. The experiment revealed that the method could not identify a 4-fold dilution in the density of cells stimulated by concanavalin A. However, lectin-induced blastogenesis involves complex cell interactions, and such a dilution may not lead to a reduction in the amount of blastogenesis obtained, since the degree of tritiated-thymidine incorporation need not be a linear function of the density of cells even under ideal conditions. Whatever the reason for the insensitivity of the assay, the fact that it is insensitive vitiated its use for the purpose intended.

A possible explanation for the inconsistency in my results with antigen may be the existence of "non-responders" and "responders" within the inbred PVG rat population, since a common feature of all experiments was a significant between-rat-within-treatment variation. On the other hand, this variability also occurred among the uninfected rats. The obvious way to overcome this problem would be to use more rats per experiment but, logistically, this was not a realistic alternative. The comparative study of lymphocyte transformation originally planned proved to be impossible, but the experiments performed did indicate a number of areas where this assay could be exploited to provide further information on the general properties of the cellular response to *Strongyloides spp.*

## 10.4. Homologous vs heterologous challenge

Results of the cross-challenge infections, where rats were primed with either a full infection or immune cells, gave information on the antigenic relationship of the two strains of *S.ratti* and *S.venezuelensis*. For reasons already explained it was not possible to test all the parasite combinations intended. The outcome of these experiments is discussed as follows:-

a) the results of challenge using full infections to prime animals

b) the results of the adoptive transfer experiments

c) an integrated view showing how the results of (a) and (b) can fit into a theory on the antigenic relationship of the three parasites.

Initial studies showed that priming rats with an exact dose of 91-98 third-stage *S.ratti* larvae elicited a strong protective response against homologous challenge with a similar small dose. This response was expressed as a reduction in the proportion of the challenge dose recovered, and a reduction in the number of eggs *in utero* per worm, compared with secondary controls (page **93**). The reduction in both parameters over the course of a primary infection of *S.ratti* (see earlier) may well have been due to an anti-worm response by the host. Previous workers, using a priming and challenge dose 10 times higher than that used in these experiments, have also demonstrated a resistance to re-infection with *S.ratti* (Sheldon, 1937a, 1937b; Moqbel and Denham, 1977, 1978; Moqbel and McLaren, 1980). Dawkins and Grove (1982) found that prior infection with as few as 6 third-stage larvae of a homogonic strain of *S.ratti* could protect mice against homologous challenge infection with 500 infective larvae. The criteria of these workers were

reduction in the number of third-stage larvae produced per gm faeces, and a 97% reduction in the worm burden, compared with controls.

The present results of reciprocal cross-immunity experiments involving both strains of *S.ratti* showed an asymmetry, since, if the heterogonic strain was used to prime rats, homologous challenge produced a significantly greater relative reduction in the proportion of the dose recovered as well as in the number of eggs *in utero* per worm (page 105). However, if the homogonic strain was the priming agent, there was no difference in the level of immunity elicited by homologous and heterologous challenge.

Prior infection with an exact dose of less than 100 third-stage *S.venezuelensis* larvae failed to immunise adult rats against homologous, or heterologous challenge with *S.ratti* (page 100) probably because the number of *S.venezuelensis* adults present post-primary infection was below the threshold necessary to elicit an immune response. It has been demonstrated in other systems that a threshold to the immune response does exist. Wortis *et al.* (1969), investigating the antibody response of mice primed against sheep erythrocytes, found that they were unable to detect any immunoglobulin A plaque forming cells in the spleen cells of mice immunised with less than $4x10^8$cells. Similarly, Askonas and Rhodes (1965) found that the minimum amount of haemocyanin necessary to give a significant antibody response above control levels was 0.001µg/mouse in primed mice. The "threshold" theory is supported by the fact that adult *S.venezuelensis* worms were recovered from the heterologous challenged rats (page 103) showing that a protective response was not expressed against the primary infection with this species. Thus challenging *S.ratti* worms simply augmented the *S.venezuelensis* already present. Primary infection *S.venezuelensis* were

probably also present in the homologous challenge treatment in both experiments but, with my experimental protocol, it was impossible to distinguish adults from the primary and secondary infection. It should be emphasized that this problem arose only in the few cases discussed. In the general framework of this study remnants of the primary infection did not complicate the interpretataion of results after challenge.

Although rats were not challenged until day 28 post-infection, it was still not shown that the immunity revealed by such experiments was of a specific or non-specific origin, since it was quite possible that the inflammatory response against the primary infection had not totally subsided. However, it was possible to transfer immunity against homologous and heterologous challenge using immune mesenteric lymph node cells primed against any one of the three parasites; demonstrated as a reduction in the proportion of the dose recovered and in the number of eggs *in utero* per worm of the challenged treatments compared with their relevant controls (page 166 and 167). The one exception to this pattern was the heterologous treatment with homogonic *S.ratti* in Expt 12 for, in that case, the difference in eggs *in utero*, though consistent with other results, was not significant (page 167). In a replicate experiment, however, a significant reduction in this parameter did occur in the relevant treatment (page 167). The small discrepancy cannot outweigh the general conclusion from these experiments that the cross-protection found previously had a specific origin.

Quantitative comparison of the level of protection produced after homologous and heterologous challenge showed that the results of the cell transfer experiments mirrored those carried out using full infections (see earlier). There was án asymmetery in the resistance expressed against

homologous and heterologous challenge with *S.ratti* which depended on the strain used to prime rats. If the homogonic strain was used, there was no significant difference in the level of immunity expressed against homologous and heterologous challenge. In contrast, if the heterogonic strain was used a greater immunity was expressed against homologous challenge. This emerged as a significantly greater relative reduction in the worms in Expt 10 and eggs in Expt 11 (page 168 and 169)

The results of the experiments where *S.venezuelensis* was used to prime cell donors confirmed that there was an antigenic difference between the two *S.ratti* strains. If the egg data were used to assess the level of protection, then there seemed to be a graded immune response against the three parasites (page 171). Homologous challenge produced the greatest level of immunity, followed by heterologous challenge with the heterogonic strain of *S.ratti*, and minimum immunity was expressed after challenge with homogonic *S.ratti*.

From these results it is apparent that the three parasites possess common functional antigens, and that there is more cross-reaction between heterogonic *S.ratti* and *S.venezuelensis* than between homogonic *S.ratti* and *S.venezuelensis*. Results following the challenge of rats primed at three weeks of age with third-stage larvae of *S.venezuelensis* are in agreement (see earlier).

Hypotheses

The asymmetry in the host's immune response against *S.ratti* could be explained by one of two theories:-

a) Antigenic polymorphism within the populations of both strains

Taking the simplest model, we might suppose that the same antigen locus exists as two mutually exclusive polymorphs "x" and "y" (Fig.10:5, page 253). If populations of worms of the homogonic strain had a ratio of "x worms" to "y worms" of 4:1, priming with this strain could feasibly give enough stimulation for the host to produce a protective response against both "x" and "y" worms. The imbalance could then reside in the possibility that virtually all the heterogonic strain individuals expressed the "x" determinant. Thus, priming with the homogonic strain would protect against "x worms" from either strain (i.e. all heterogonic and 80% of the homogonic worms), as well as "y worms" within the homogonic strain itself (the remaining 20% of that population). Priming with the heterogonic strain would only protect the host against the "x worms", with the result that 20% of the homogonic strain expressing the "y" polymorph would escape the host's immune response.

b) Existence of a strain-specific antigen

If a strain-specific antigen was responsible for the asymmetry of the host's immune response (Fig.10:5, page 253) various assumptions would have to be made to explain the imbalance. i) worms of both strains must express common functional antigens in order to obtain a cross-protective response in reciprocal challenge experiments

ii) the proportion of strain-specific functional antigen(s) must be low compared to the common antigens, or there would be no asymmetry.

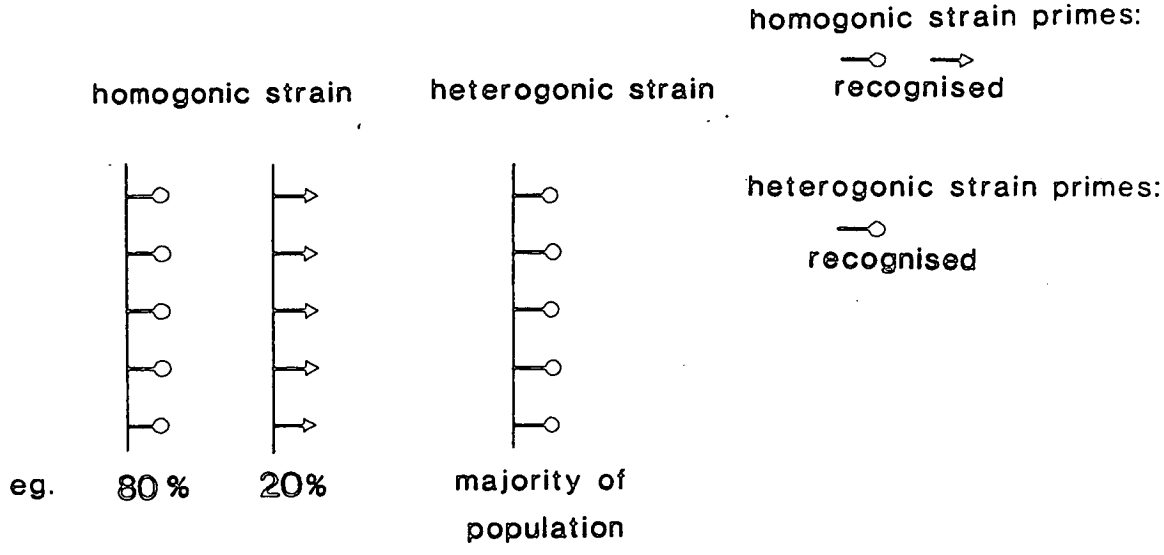
iii) the locus of the strain-specific antigen must be either on a common organ system or in excretory/secretory products.

iv) this model would have to assume that the two strain-specific antigen(s) either varied in their relative immunogenicity, perhaps due to a different size,

Fig.10:5

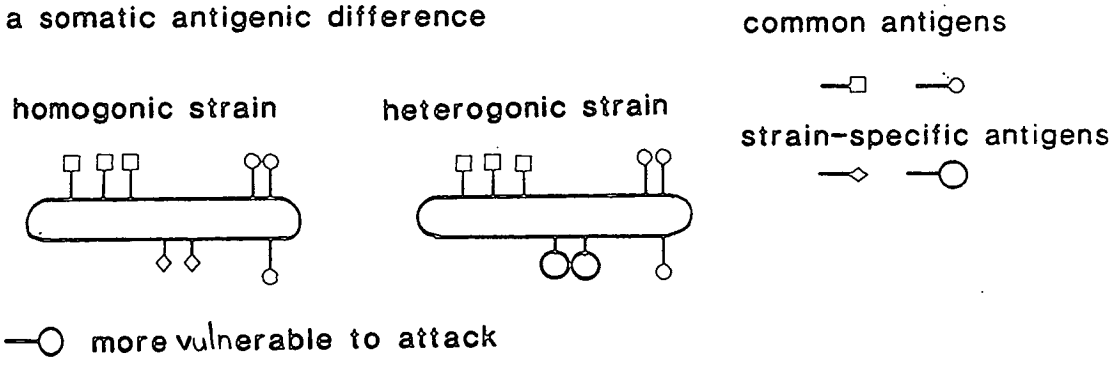
Theories to explain results of cross-immuity studies using two strains of S.ratti

a) Antigenic polymorphism among individuals




b) Strain-specific antigen

eg. a somatic antigenic difference



charge or configuration; or that the attack of one had a more debilitating effect on the worms' survival.

v) the heterogonic strain would have to be more vulnerable to attack.

If rats were primed with either strain the host would recognise the common functional antigens and produce an immune response against both parasites. If the homogonic strain was used to prime rats the host would also recognise the homogonic strain-specific antigen, but since a response directed against the antigen had little detrimental effect, both parasites would be equally affected. In contrast, priming with the heterogonic strain, would produce a more severe response against heterogonic worms compared with that directed against homogonic adults and thus a greater immunity would be demonstrated after homologous challenge.

One way to test these theories would be to culture homogonic strain infective larvae from successive generations of challenge infections of animals primed with the heterogonic strain. If the first theory applies this should select for a population of homogonic worms containing proportionally more adults expressing the "y" polymorph. One would expect a stepwise decline in reciprocal cross-immunity between the two strains after each passage. If the second theory applies then the proportion of the worms recovered from heterologous challenged rats should remain constant. Another method which could be used would be to try and characterize the antigenic differences between the strains. If the second theory applied the two strains could be separated on the basis of the strain-specific antigen they expressed. However, if the first theory applies, the heterogeneity would be between individuals of the homogonic strain and not between the strains.

One of the difficulties with the first theory is explaining how the polymorphism is maintained within the parasite population. From present knowledge, the conventional mechanism for selection based on genetic change seems to be lacking in *Strongyloides spp.* In particular, genetic recombination of the classical kind is probably absent since, in those species investigated sufficiently well, both parasitic and free-living females reproduce parthenogenetically (Zaffagnini, 1973; Bolla and Roberts, 1968; Albertson *et al*, 1979). Despite this, the genome clearly possesses some flexibility, since strains with completely homogonic development can be artificially selected from heterogonic varieties (Sandground, 1926). It is possible that a high mutation rate, or extra-nuclear events (since the sperm does enter the egg in the free-living cycle, even though nuclear fusion fails), maintain the diversity. It is relatively simple to visualise an underlying mechanism in support of the second theory, since a chance mutation in one individual could introduce the strain-specific antigen into the population. This would initiate a new clone of parasites in the absence of the normal, sexual, means of maintaining the gene pool. The only way to answer this question would be to carry out an in depth study of the genetic structure of the two strains.

### 10.5. Conclusions

Most workers studying nematode immunology use large doses, which may create an atypical immune response whose very intensity could complicate an analysis of contributing processes, specific and non-specific. Among the former, large doses may lead to abnormal ratios of T cell subsets, and thus seriously distort the outcome compared with infections of more "natural" proportions. The present results clearly showed that specific immunological events are important in directing the responses which cause parasite

expulsion, since resistance could be transferred with immune cells and specificity was expressed by the host's immune response elicited at challenge. It may be worthwhile to carry out in depth investigations on the contribution of specific components of the host's immune system in parasite rejection since, at present, most of the effort in laboratories devoted to experimental immunology of nematode infection is concentrated on the role of non-specific mechanisms (see Introduction). Moreover, the experimental system developed in this work, particularly in regard to the asymmetry it reveals, could be exploited further to clarify the inter-relationships of the two types of process.

CHAPTER 11

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12.

# APPENDIX

Appendix 1:1
Radio-active labels used in this study

I) L-[$^{75}$-Se] Selenomethionine
Concentration:37MBq/ml. (Amersham International Ltd.)
Stored at +4°C
tl/2 =118.5 days decays at 100% electron capture to stable arsenic
Working Concentration = 74kBq/ml. made up with distilled water

II). [methyl-$^{3}$H] thymidine
Concentration:37Mbq/ml. (Amersham International Ltd.)
Stored at +4°C
tl/2 = 12.26 years
Working Concentration = 925kBq/ml. made up with serum-free medium (see Appendix 1:2, page )

Appendix 1:2
Compounds for adoptive transfer

I)Cell medium

| | | | |
|---|---|---|---|
| penicillin/streptomycin | 10,000 units/ml. | (Gibco) | |
| human serum | 15% heat-inactivated | | |
| RPMI-1640 with 25mM hepes with L-glutamine | | (Gibco) | (see below) |
| L-glutamine | 20mM(x200) | (Gibco) | |
| heparin | | | |

100 ml medium
Solution A was made by adding 5ml of penicillin/streptomycin and 5ml of L-glutamine to 500ml of RPMI-1640. 100ml of cell medium was prepared by adding 85ml of A to 15 of human serum, which had been heat-inactivated for 45 minutes.

II)Ethidium bromide/acridine orange
100x stock solution

| | |
|---|---|
| 50mg. ethidium bromide | (Sigma) |
| 15mg. acridine orange | (BDH) |

The above powders were dissolved oi 1ml. of 95% ethanol and 49ml of distilled water was added. The solution was mixed well and stored at -20°C in 1ml aliquots

1x solution
1ml was made up 1:100 with phosphate buffered saline (see Appendix 1:4) and stored in an amber bottle at +4°C. The solution was active for one month.

Appendix 1:3
Solutions for lymphocyte transformation assay

I) Cell medium

The medium was essentially the same as that used for adoptive transfer (see Appendix 1:2) with the addition of beta-mercaptoethanol ($5x10^{-5}$ M, Sigma ), 50µl/100ml of medium, and the exclusion of heparin.

II) Concanavalin A (Sigma, type 5, lyophilised)

Normally 2.5µg/ml. concentration was used, which was made up with serum-free medium (from above), and then filter sterilised by passing through a 0.22µm. filter (Millipore). It was made up x3 the final concentration needed to allow for the 1:3 dilution in the well of the micro-titre plate.

III) Scintillant

| | | |
|---|---|---|
| Toluene | | (Mackay and Lynn) |
| PPO [2,5 -diphenyloxazole] | 6gm./litre | (Sigma) |
| POPOP [(1,4 bis <2-(5 phenylozozolyl)) benzene)] | 0.05gm/litre | (Sigma) |

These powders were dissolved in 1 litre of toluene.

IV) Giensa stain

Giemsa (Gurr) was made up to 10% with Giemsa buffer, pH 7.3 (Gibco)

Appendix 1:4

I). Compounds for antigen production

Adult culture medium

| | | |
|---|---|---|
| RPMI-1640 with 25mM hepes with L-glutamine | | (Gibco) |
| penicillin/streptomycin (10,000 units/ml) | 2ml/100ml of medium | (Gibco) |
| L-glutamine 20mM (x200) | 1ml/100ml of medium | (Gibco) |
| sodium pyruvate | 10% volume | (Gibco) |
| Fungizone® lyophilized (250mcg/ml) | 2ml/100ml of medium | (Gibco) |

100ml of the medium was made using the above constituents, made up to 100ml with RPMI-1640, and it was stored at +4°C until needed.

| | | | |
|---|---|---|---|
| II). Phosphate buffered saline | pH 7.3 | Dulbecco A | (Oxoid Ltd.) |
| disodium hydrogen phosphate | 1.15gm/litre | | |
| sodium chloride | 8gm/litre | | |
| potassium dihydrogen phosphate | 0.2gm/litre | | |
| potassium chloride | 0.2gm/litre | | |

III). Supplemented phosphate buffered saline

1ml. of penicillin/streptomycin (10,000 units/ml, Gibco) was added to 100ml of phospate buffered saline

IV). Polyethylene glycol 6000 (BDH)

III) RPMI-1640

| Inorganic salts | mg/ml |
|---|---|
| hydrated calcium nitrate | 100 |
| potassium chloride | 400 |
| hydrated magnesium sulphate | 100 |
| sodium chloride | 6000 |
| sodium hydrogen carbonate | 2000 |
| hydrated sodium hydrogen phospate | 1512 |

| Other compounds | |
|---|---|
| glucose | 1 |
| glutathione(reduced) | 5 |

| Amino-acids | |
|---|---|
| L-arginine hydrogen chloride | 50 |
| L-asparagine | 20 |
| L-cystine | 50 |
| L-glutamic acid | 20 |
| L-glutamine | 300 |
| glycine | 10 |
| L-histidine hydrogen chloride $H_2O$ 15 | |
| L-hydroxyproline | 20 |
| L-isoleucine(allo free) | 50 |
| L-leucine(methionine free) | 50 |
| L-lycine hydrogen chloride | 40 |
| L-methionine | 15 |
| L-phenylalanine | 15 |
| L-proline(hydroxy L-proline free) | 20 |
| L-serine | 80 |
| L-threonine(allo free) | 20 |
| L-tryptophan | 5 |
| L-tyrosine | 20 |

| Vitamins | |
|---|---|
| biotin | 0.200 |
| D-calcium pantothenate | 0.200 |
| choline chloride | 3.000 |
| folic acid | 1.000 |
| i-inositol | 35.000 |
| nicotinamide | 1.000 |
| para-aminobenzoic acid | 1.000 |
| pyridoxine hydrogen chloride | 1.000 |
| riboflavin | 0.200 |
| thiamine hydrogen chloride | 1.000 |
| vitamin $B_{12}$ | 0.005 |

Appendix 1:5
Compounds for Micro-Lowry
Solutions

| | | |
|---|---|---|
| 2% sodium carbonate | (Analar, BDH Chemicals) | "A" |
| Ø.5% hydrated copper sulphate | (Sigma) | "B" |
| 1% sodium/potassium tartrate | (Sigma) | "D" |

I).Solution D
19.2ml of "A" was added to Ø.4ml of "B" and Ø.4ml of "C"

II).Solution E
Folin and Ciocalteu's phenol reagent (Fisons) 1:1 with water

III).Trichloroacetic acid 25% (Sigma) Stored at +4°C

IV).Sodium hydroxide(Sigma) 1M Stored at room-temperature

V).Albumin, bovine(Fraction 5,Sigma)
BSA was made up to various concentratios using phosphate buffered saline (see Appendix 1:4, part II).

Appendix 1:6
Background counts over the course of lymphocyte transformation tests

| Date measured | Mean counts/min | n | SD |
|---|---|---|---|
| 19.6.84 | 15 | 5 | 3.Ø |
| 24.7.84 | 16 | 5 | 3.8 |
| 1.11.84 | 16 | 5 | 2.1 |
| 2Ø.1.85 | 18 | 2Ø | 2.5 |
| 19.2.85 | 16 | 5 | 2.7 |
| 28.7.85 | 14 | 5 | 2.8 |
| 5.8.85 | 15 | 14 | 2.4 |
| 9.8.85 | 15 | 5 | 2.3 |
| overall mean: | 16 | 64 | 3.Ø |

Appendix 1:7

Correcting two-level nested anova tables used to analysis egg data for unequal sample sizes

Initially coefficients of variance were calculated from quantities 1-4.

$$\text{Quantity 1} = \sum a \sum bi\, n_{ij}$$

$$\text{Quantity 2} = \sum a \sum bi\, n^2_{ij}$$

$$\text{Quantity 3} = \sum a \left[ \sum bi\, n_{ij} \right]^2$$

$$\text{Quantity 4} = \sum a \left[ \frac{bi\, n^2_{ij}}{\sum bi\, n_{ij}} \right]$$

$$n'o = \frac{\text{Quantity 4} - [\text{ Quantity 2} / \text{Quantity 1 }]}{\text{df. Groups}}$$

$$n_o = \frac{\text{Quantity 1} - \text{Quantity 4}}{\text{df Subgroups}}$$

$$(nb)_o = \frac{\text{Quantity 1} - [\text{ Quantity 3} / \text{Quantity 1 }]}{\text{df. Groups}}$$

Using these coefficients of variance the new MS'gps. and MS' subgps. could be calculated:-

$$\text{MS gps.} = S^2 + n'o\, S^2_{BcA} + (nb)_o\, S^2_A$$

$$\text{MS subgps.} = S^2 + n_o\, S^2_{BcA}$$

Thus:-

$S^2$ = MS within, the error variance from the two-level nested anova

$$S^2_{BcA} = \frac{\text{MS subgps} - \text{MS within}}{n_o}$$

$$S^2_A = \frac{\text{MS gps} - \text{MS within} - n'o S^2_{BcA}}{(nb)_o}$$

For an approximate test of significance a new denominator mean square MS' subgps can be calculated.

$$F_s = \frac{MS\ gp}{MS'subgps} = \frac{S^2 + n'o\ S^2BcA_2 + (nb)_o\ S^2A}{S^2 + n'o\ S^2BcA}$$

Now a new critical value of F against which to test $F_s$ is needed. The degrees of freedom for MS gps are unchanged but the denominator is a new mean square with new degrees of freedom, df' subgps. To compute these a new MS' subgps. is calculated in terms of the original mean square.

$$MS'\ subgps = S^2 + n'o\ S^2BcA$$

$$= MS\ within + n'o\ \left[\frac{MS\ subgps}{n_o}\right]$$

$$= \left[1 - \frac{n'o}{n_o}\right] MS\ within + \frac{n'o}{n_o} MS\ subgps$$

A general formula for the new df' of a reconstituted mean square MS' is:-

$$df' = \frac{(MS')^2}{\frac{i(wi\ MSi)^2}{dfi}}$$

where "wi" are the coefficients of the mean squares, df' are the original degrees of freedom of the mean squares MSi, and i indicates summation over all the constituent mean squares. Thus for df' subgps. :-

$$df'\ subgps = \frac{(MS\ subgps)^2}{\frac{[(1 - \frac{n'o}{n_o} \times MS\ within)^2]}{df\ error} + \frac{[(\frac{n'o}{n_o} \times MS\ subgp)^2]}{df\ subgps}}$$

This is obviouly quite a complicated process and very time consuming. A few examples were carried out to find out how much of a difference there was between the original MS gps and MS subgps and the newly calculated MS' gps and MS' subgps, and to the original F ratio obtained and the newly calculated F' ratio. In both cases there was little difference, therefore the results were not corrected for unequal sample sizes ( see examples 1 and 2 below ).

Example 1
Expt. 6 (Chapter 5)

Original anova table

| Source Of Variation | df | SS | MS | $F_s$ | |
|---|---|---|---|---|---|
| Among Gps | 4 | 1068.274 | 267.069 | 112.12 | |
| Among Subgps | 23 | 54.788 | 2.382 | 1.44 | |
| Within | 234 | 388.022 | 1.658 | | |
| Total | 261 | 1511.084 | 5.790 | | |

Newly calculated anova table (correcting for unequal sample sizes)

| Sources Of Variation | df | SS | MS' | $F_s$' | |
|---|---|---|---|---|---|
| Among Gps | 4 | 1068.274 | 284.77 | 119.15 | |
| Among Subgps | 20 | 54.788 | 2.39 | 1.44 | |
| Within | 234 | 388.022 | 1.66 | | |
| Total | 261 | 1511.084 | | | |
| $F_{(4,20)}$ = 7.10 | 0.1% | | $F_{(12,\infty)}$ = 1.75 | 5% | |

The method of calculating the new MS' and df' is shown below:-

$$\text{Quantity 1} = \sum^{a} \sum^{bi} n_{ij} = 13+12+14+ \ldots\ldots +20+6 = 262$$

$$\text{Quantity 2} = \sum^{a} \sum^{bi} n^2_{ij} = 13^2+12^2+\ldots 6^2 = 3324$$

$$\text{Quantity 3} = \sum^{a} [\sum^{bi}]^2 = 54^{20}+32^2+\ldots 101^2 = 18578$$

$$\text{Quantity 4} = \sum^{a} \left[ \frac{\sum^{bi} n^2_{ij}}{\sum^{bi} n_{ij}} \right] = \frac{13^2+ 12^2+\ldots+15^2}{54}$$

$$= 52.24$$

$$n'_o = \frac{\text{Quantity 4} - [\text{Quantity 2} / \text{Quantity 1}]}{\text{df gps}}$$

$$= \frac{52.24 - [\ 3324 / 262\ ]}{4}$$

$$= 9.89$$

$$n_o = \frac{\text{Quantity 1} - \text{Quantity 4}}{\text{df subgps}}$$

$$= \frac{262 - 52.24}{23}$$

$$= 9.12$$

$$(nb)_o = \frac{\text{Quantity 1} - [\text{Quantity 3}/\text{Quantity 1}]}{df}$$

$$= \frac{262 - [18578/262]}{4}$$

$$= \underline{47.77}$$

$$S^2 = \text{MS within} = \underline{1.66}$$

$$S^2BcA = \frac{\text{MS subgps} - \text{MS within}}{n_o} = \frac{2.38 - 1.66}{9.12} = \underline{\quad .08}$$

$$S^2A = \frac{\text{MS gp} - \text{MS within} - n'o\ S^2BcA}{(nb)_o}$$

$$= \frac{267.07 - 1.66 - (9.89)(.08)}{47.77}$$

$$= \underline{5.91}$$

$$F_s = \frac{\text{MS gp}}{\text{MS' subgps}} = \frac{S^2 + n'o\ S^2\ BcA + (nb)_o\ S^2\ A}{S^2 + n'o\ S^2\ BcA}$$

$$= \frac{1.66 + 9.89(.08) + 47.77(5.91)}{1.66 + 9.89(.08)}$$

$$= \frac{284.77}{2.45}$$

$$= \underline{116.23}$$

$$\text{MS' subgps} = (1 - \frac{n'o}{n_o})\text{MS within} + \frac{n'o}{n_o}\text{MS subgp}$$

$$= -.08(1.66) + 1.08(2.38)$$

$$= \underline{2.39}$$

$$df'\ \text{subgp} = \frac{2.39^2}{[(\frac{-.08 \times 1.66}{234})^2] + [(\frac{1.08 \times 2.38}{23})^2]}$$

$$= \frac{5.71}{.29}$$

$$= \underline{19.7}$$

Example 2 (Expt. 8, Chapter 5, page )

Original anova table

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps | 1 | 102.145 | 102.145 | 20.91 |
| Among Subgps | 13 | 63.487 | 4.884 | 2.50 |
| Within | 225 | 439.102 | 1.952 | |
| Total | 239 | 604.733 | | |

Newly calculated anova table (corrected for unequal sample sizes )

| Sources Of Variation | df | SS | MS' | F ' | |
|---|---|---|---|---|---|
| Among Gps | 1 | 102.145 | 102.38 | | 23.37 |
| Among Subgps | 14 | 63.487 | 4.41 | | 2.26 |
| Within | 225 | 439.102 | 1.952 | | |
| Total | 239 | 604.733 | | | |

$F_{(1,14)}$ = 17.14 0.1% $F_{(12,\infty)}$ = 2.18 1%

The new mean squares and df' were calculated as shown below:-

Quantity 1 = 240
Quantity 2 = 4204
Quantity 3 = 38,322
Quantity 4 = 31.02

$$n'o = \frac{31.02 - [\ 4204 / 240\ ]}{1} = 13.50$$

$$(nb)_o = \frac{240 - (\ 38,322 / 240\ )}{1} = 80.33$$

$$n_o = \frac{240 - 31.02}{13} = 16.08$$

MS Within $= S^2 = 1.952$

$$S^2 \text{ BcA} = \frac{4.884 - 1.952}{16.08} = 0.18$$

$$S^2 \text{ A} = \frac{102.145 - 1.952 - 13.5(0.18)}{80.33}$$

$$= 1.22$$

$$F_s = \frac{1.952 + 13.5(0.18) + 80.33(1.22)}{1.952 + 13.5(0.18)}$$

$$= 23.37$$

$$\text{MS' subgps} = [\ 1 - \frac{13.50}{16.08}\ )1.952 + \frac{13.50}{16.08}(4.884)$$

$$= 4.41$$

$$df' \text{ subgps} = \frac{(4.41)^2}{\left[\frac{(0.16 \times 1.952)^2}{225}\right] + \left[\frac{(0.84 \times 4.884)^2}{13}\right]}$$

$$= \underline{13.99}$$

Appendix 1:8
The method of calculating the resistance ratios and resistance quotients

For simplicity the method for calculating the resistance ratios and the resistance quotients for the worm data only is shown, the method is identical for the egg data, but " the mean number of eggs in utero/worm " should be substituted for "the mean proportion of the dose recovered". Data from experiment 7, chapter 4 , (see page ), was used in this example, where rats were primed with an exact dose of less than 100 third-stage heterogonic S.ratti larvae, and challenged with a similar small dose of the homologous or heterologous strain.

a) Homologous Challenge

| Proportion Of the Dose Recovered from Each Exptl. Rat | Mean Proportion Of the Dose Recovered from Controls | Resistance Ratio *1 | Resistance Quotient ReQw*2 |
|---|---|---|---|
| 0.000 | 0.368 | 0.000 | 1.000 |
| 0.000 | 0.368 | 0.000 | 1.000 |
| 0.000 | 0.368 | 0.000 | 1.000 |
| 0.000 | 0.368 | 0.000 | 1.000 |
| 0.020 | 0.368 | 0.054 | 0.946 |
| 0.010 | 0.368 | 0.027 | 0.973 |
| 0.031 | 0.368 | 0.084 | 0.916 |
| 0.030 | 0.368 | 0.082 | 0.918 |
| 0.000 | 0.368 | 0.000 | 1.000 |
| 0.010 | 0.368 | 0.027 | 0.973 |
| | n | 10 | 10 |
| | Mean | 0.027 | 0.973 |
| | SD | 0.034 | 0.034 |

Where:-

*1 Resistance Ratio, $R = \frac{a}{b}$

a = proportion of the dose recovered for each experimental rat
b = mean proportion of the dose recovered from the relevant control

*2 Resistance Quotient, ReQ = 1 - R

For example, for observation 4, where the proportion of the dose recovered for the experimental rat was 0.020 then:-

$$\text{Resistance Ratio} = \frac{0.020}{0.368} = 0.054$$

$$\text{Resistance Quotient ReQw} = 1 - 0.054 = 0.946$$

This procedure was followed for each individual observation within the treatment and then repeated for the results of the heterologous challenged group in (b)

b) Heterologous Challenge

| Proportion Of the Dose Recovered for Each Exptl Rat | Mean Proportion Of the Dose Recovered for Controls | Resistance Ratio | Resistance Quotient ReQw |
|---|---|---|---|
| Ø.Ø2Ø | Ø.524 | Ø.Ø38 | Ø.962 |
| Ø.168 | Ø.524 | Ø.321 | Ø.679 |
| Ø.265 | Ø.524 | Ø.5Ø6 | Ø.494 |
| Ø.168 | Ø.524 | Ø.321 | Ø.679 |
| Ø.167 | Ø.524 | Ø.319 | Ø.681 |
| Ø.Ø4Ø | Ø.524 | Ø.Ø76 | Ø.924 |
| Ø.237 | Ø.524 | Ø.452 | Ø.548 |
| Ø.177 | Ø.524 | Ø.338 | Ø.662 |
| Ø.265 | Ø.524 | Ø.5Ø6 | Ø.494 |
| Ø.Ø85 | Ø.524 | Ø.162 | Ø.838 |
| | | n 1Ø | 1Ø |
| | | Mean Ø.3Ø4 | Ø.696 |
| | | SD Ø.166 | Ø.166 |

Two things become apparent from (a) and (b) :-

(i) the mean resistance ratio and the mean resistance quotient have the same "n" and standard deviation, and thus the same standard error.

(ii) the mean resistance qoutient can be calculated directly from the mean resistance ratio, i.e.:-

Mean Resistance Quotient = 1 - [ Mean Resistance Ratio]

for example, if the results given in (a) are considered then:-

Mean Resistance Ratio = Ø.Ø27

thus Mean Resistance Quotient = 1 - Ø.Ø27 = Ø.973

from (a) Mean Resistance Quotient = Ø.973

## 2:1 Summary of results for Section 3:1

Table 2:1(i)
The effect of pairing rats on the proportion of the homogonic S.ratti dose recovered, Expt 1

| Treatment | Mean proportion of the dose recovered | n | SD | Mean exact dose |
|---|---|---|---|---|
| single | 0.247 | 14 | 0.116 | 92.53 |
| paired | 0.223 | 16 | 0.156 | |
| injection controls (day 8) | 0.548 | 6 | 0.112 | 89.00 |

Table 2:1(ii)
Distribution of homogonic S.ratti worms along the intestine in Expt 1

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| single | Mean | 0.37 | 0.33 | 0.16 | 0.09 | 0.02 | 0.02 | 0.01 | 0.01 | 15 |
| | SD | 0.08 | 0.11 | 0.08 | 0.06 | 0.03 | 0.03 | 0.02 | 0.02 | |
| | mean no. of worms per rat 22 | | | | | | | | | |
| paired | Mean | 0.40 | 0.36 | 0.17 | 0.03 | 0.02 | 0.00 | 0.001 | 0.003 | 15 |
| | SD | 0.25 | 0.16 | 0.13 | 0.05 | 0.05 | 0.00 | 0.004 | 0.011 | |
| | mean no. of worms per rat 20 | | | | | | | | | |
| injection controls | Mean | 0.35 | 0.35 | 0.20 | 0.09 | 0.02 | 0.004 | 0.00 | 0.00 | 6 |
| | SD | 0.04 | 0.03 | 0.03 | 0.04 | 0.02 | 0.010 | 0.00 | 0.00 | |
| | mean no. worms of per rat 48 | | | | | | | | | |

## 2:2 Summary of results Section 3:2

Table 2:2(i)
An attempt to demonstrate "self-infection" in Rats, homogonic S.ratti Expt 2

| Treatment | Mean proportion of the Dose recovered | n | SD |
|---|---|---|---|
| infected "A" | 0.340 | 7 | 0.119 |
| infected "B" | 0.403 | 7 | 0.177 |
| uninfected "A" | 0.000 | 7 | 0.000 |
| uninfected "B" | 0.000 | 6 | 0.000 |
| injection controls | 0.543 | 6 | 0.104 |
| mean exact dose | skin-application | | 90.14 |
| | subcutaneous injection | | 87.17 |

Where:-

"A" - rats clenaed out every two days

"B" - rats cleaned out once on day 10 post-infection

Table 2:2(ii)
Distribution of homogonic S.ratti worms in Expt 2

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| injection controls | Mean | 0.31 | 0.32 | 0.16 | 0.16 | 0.04 | 0.01 | 0.003 | 0.00 | 6 |
| | SD | 0.06 | 0.04 | 0.08 | 0.07 | 0.07 | 0.02 | 0.010 | 0.00 | |
| | mean no. of worms per rat 47 | | | | | | | | | |
| infected rats (pooled) | Mean | 0.36 | 0.37 | 0.18 | 0.08 | 0.03 | 0.004 | 0.00 | 0.001 | 14 |
| | SD | 0.16 | 0.11 | 0.09 | 0.05 | 0.05 | 0.010 | 0.00 | 0.004 | |
| | mean no. of worms per rat 33 | | | | | | | | | |

2:3 Summary of results from Section 3:3

Table 2:3(i)
Number of worms recovered during incubation of intestine, Expt 3

| Parasite | Day post-infection | Time in saline digest in minutes 90 | 120 | 180 | 240 | 300 | Total no. worms recovered | Mean prop. of the dose recovered | SD |
|---|---|---|---|---|---|---|---|---|---|
| S.venezuelensis | 7 | 0 | 290 | 65 | 48 | 14 | 417 | 0.321 | 0.13 |
| | | 73 | 83 | 44 | 30 | 0 | 230 | | |
| homogonic S.ratti | 7 | 22 | 160 | 60 | 145 | 0 | 387 | 0.378 | 0.01 |
| | | 15 | 240 | 80 | 34 | 0 | 369 | | |
| heterogonic S.ratti | 7 | 56 | 220 | 41 | 61 | 0 | 378 | 0.347 | 0.04 |
| | | 0 | 140 | 110 | 5 | 60 | 315 | | |

Table 2:3(ii)
The effect of time spent in the extra-intestinal environment on the ability of worms to establish in a new host, homogonic S.ratti, Expt 4

| Treatment | Mean prop. of the dose Recovered | n | SD | Mean no. eggs in utero/ worm/rat | n | SD | Mean no. eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| 1 Hour | 0.516 | 4 | 0.156 | 6.875 | 4 | 0.66 | 6.734 | 37 | 1.561 |
| 2 Hours | 0.050 | 4 | 0.042 | 5.331 | 1 | 0.00 | 5.333 | 3 | 1.155 |
| 3 Hours | 0.182 | 4 | 0.158 | 6.565 | 3 | 0.44 | 6.375 | 16 | 1.028 |
| 4 Hours | 0.174 | 4 | 0.149 | 7.417 | 2 | 0.83 | 7.222 | 9 | 1.641 |
| mean exact dose | | | 49.13 | | | | | | |

Table 2:3(iii)
The effect of varying the donor of adult worms on their ability to survive post-transfer, S.venezuelensis, Expt 5

| Treatment | Mean proportion of the dose recovered | n | SD | Mean exact dose |
|---|---|---|---|---|
| Donor A | 0.311 | 4 | 0.213 | |
| Donor B | 0.151 | 3 | 0.117 | 47.75 |
| Donor C | 0.380 | 4 | 0.116 | |
| Donor D | 0.249 | 4 | 0.112 | |
| overall mean | 0.281 | 15 | 0.165 | |

Table 2:3(iv)
Distribution of S.venezuelensis worms along the intestine in Expt 4

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 Hour | Mean | 0.69 | 0.28 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.30 | 0.26 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 25 | | | | | | | | |
| 2 Hours | Mean | 0.93 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.12 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 2 | | | | | | | | |
| 3 Hours | Mean | 0.78 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.20 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 8 | | | | | | | | |
| 4 Hours | Mean | 0.65 | 0.31 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.47 | 0.47 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 8 | | | | | | | | |

Table 2:3(v)
Distribution of S.venezuelensis worms along the intestine in Expt 5

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| Donor A | Mean | 0.89 | 0.04 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.29 | 0.08 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 15 | | | | | | | | |
| Donor B | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 7 | | | | | | | | |
| Donor C | Mean | 0.90 | 0.09 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.10 | 0.12 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 18 | | | | | | | | |
| Donor D | Mean | 0.90 | 0.07 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.15 | 0.10 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 11 | | | | | | | | |
| Overall | Mean | 0.92 | 0.05 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 15 |
| | SD | 0.17 | 0.09 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 13 | | | | | | | | |



## 2:4 Summary of results from Section 3:5

Table 2:4(i)
The number of mesenteric lymph node cells recovered per rat, Expt 8

| Rat No. | No. Cells/ml. | Total No. Cells | % Dead |
|---|---|---|---|
| 1 | $3.7\times10^{8}$ | $1.11\times10^{9}$ | 26.7 |
| 2 | $4.4\times10^{7}$ | $8.8\times10^{8}$ | 53.9 |
| 3 | $1.7\times10^{7}$ | $9.2\times10^{8}$ | 0.0 |
| Mean | $1.44\times10^{8}$ | $9.7\times10^{8}$ | |
| SD | $1.96\times10^{8}$ | $1.23\times10^{8}$ | |

Table 2:4(ii)
The effect of cell transfer medium on the proportion of the homogonic S.ratti dose recovered, Expt 7

| Treatment | Mean prop. of the dose recovered | n | SD | Mean no. of Eggs in utero/ worm | n | SD | Mean exact dose |
|---|---|---|---|---|---|---|---|
| No Medium | 0.541 | 6 | 0.113 | 7.229 | 97 | 1.245 | 98.25 |
| Medium | 0.542 | 6 | 0.086 | 7.129 | 106 | 1.137 | |

Table 2:4(iii)
Distribution of homogonic S.ratti worms along the intestine in Expt 7

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| Medium | Mean | 0.42 | 0.26 | 0.18 | 0.10 | 0.03 | 0.003 | 0.00 | 0.00 | 6 |
| | SD | 0.13 | 0.09 | 0.05 | 0.04 | 0.02 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 53 | | | | | | | | |
| No Medium | Mean | 0.36 | 0.33 | 0.18 | 0.09 | 0.03 | 0.004 | 0.00 | 0.00 | 6 |
| | SD | 0.07 | 0.08 | 0.06 | 0.04 | 0.04 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 53 | | | | | | | | |

2:5 Summary of results from section 3:6

Table 2:5(i)
The effect of serum concentration in the cell medium on the level of stimulation obtained, Expt 9

| Rat No. | | Serum Concentration 5% Unstim. Control cpm | 5% Exptl. cpm | 5% Diff. Counts | 10% Unstim. Control cpm | 10% Exptl. cpm | 10% Diff. Counts | 15% Unstim. Control cpm | 15% Exptl. cpm | D. C |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Mean | 1534 | 19762 | 18228 | 1364 | 100906 | 99560 | 274 | 107647 | 10 |
| | SD | 395 | 3818 | | 399 | 18732 | | 108 | 31633 | |
| | n | 5 | 10 | | 5 | 11 | | 6 | 11 | |
| 2 | Mean | 2482 | 25478 | 22996 | 1952 | 75924 | 73972 | 378 | 99548 | 9 |
| | SD | 1318 | 3927 | | 459 | 23992 | | 181 | 10207 | |
| | n | 5 | 11 | | 6 | 12 | | 6 | 11 | |
| 3 | Mean | 489 | 32287 | 31798 | 438 | 19457 | 19019 | 240 | 32916 | 3 |
| | SD | 206 | 7722 | | 76 | 4919 | | 92 | 7089 | |
| | n | 6 | 12 | | 6 | 12 | | 6 | 11 | |

Table 2:5(ii)
The effect of Concanavalin A concentration on the level of stimulation obtained, Expt 10

| Rat No. | | Unstim. Control cpm | Concanavalin A Concentration µg/ml 20 | 10 | 5 | 2.5 |
|---|---|---|---|---|---|---|
| 1 | Mean | 1158 | 12724 | 12020 | 42120 | 123782 |
| | SD | 834 | 5712 | 1388 | 4401 | 14267 |
| | n | 9 | 11 | 11 | 5 | 11 |
| | Diff. Counts | | 11566 | 10862 | 40962 | 122624 |
| 2 | Mean | 1728 | 15677 | 16936 | 59877 | 140755 |
| | SD | 760 | 3228 | 4285 | 7391 | 23054 |
| | n | 9 | 8 | 9 | 7 | 7 |
| | Diff. Counts | | 13949 | 15208 | 58149 | 139027 |
| 3 | Mean | 852 | 14197 | 14199 | 24978 | 107030 |
| | SD | 265 | 9055 | 3781 | 3268 | 3713 |
| | n | 11 | 11 | 7 | 5 | 11 |
| | Diff. Counts | | 12469 | 12471 | 23250 | 105302 |

* Note
Diff. Count = differential count
Unstim. = unstimulated

Table 2:5(iii)
The effect of varying the day of harvesting cells on the level of stimulation obtained, Expt 11

| Rat No. | | Day Of Harvest Day 3 Unstim. Control cpm | Day 3 Exptl. cpm | Day 3 Diff. Counts | Day 4 Unstim. Control cpm | Day 4 Exptl. cpm | Day 4 Diff. Counts |
|---|---|---|---|---|---|---|---|
| 1 | Mean | 507 | 69754 | 69247 | 760 | 25775 | 25015 |
| | SD | 153 | 20896 | | 766 | 8231 | |
| | n | 5 | 11 | | 5 | 11 | |
| 2 | Mean | 300 | 81151 | 80851 | 377 | 24798 | 24421 |
| | SD | 117 | 14364 | | 227 | 1665 | |
| | n | 5 | 11 | | 5 | 10 | |
| 3 | Mean | 490 | 71552 | 71062 | 692 | 19251 | 18559 |
| | SD | 192 | 16329 | | 299 | 1736 | |
| | n | 5 | 11 | | 5 | 10 | |

Table 2:5(iv)
The effect of varying the day of harvesting cells on the level of stimulation obtained, Expt 12

| Rat No. | | Day Of Harvest Day 3 Unstim. Control cpm | Day 3 Exptl. cpm | Day 3 Diff. Counts | Day 4 Unstim. Control cpm | Day 4 Exptl. cpm | Day 4 Diff. Counts | Day 5 Unstim. Control cpm | Day 5 Exptl. cpm | Day 5 Diff. Counts |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Mean | 437 | 90121 | 89964 | 237 | 33417 | 33180 | 361 | 22974 | 22613 |
| | SD | 294 | 15517 | | 79 | 9280 | | 305 | 6835 | |
| | n | 5 | 10 | | 5 | 11 | | 5 | 10 | |
| 2 | Mean | 342 | 125777 | 125435 | 222 | 41824 | 41602 | 223 | 28237 | 28014 |
| | SD | 107 | 14017 | | 44 | 7177 | | 33 | 5410 | |
| | n | 6 | 10 | | 5 | 11 | | 5 | 10 | |
| 3 | Mean | 473 | 96710 | 96237 | 188 | 39541 | 39353 | 261 | 24623 | 24362 |
| | SD | 90 | 12449 | | 80 | 11535 | | 67 | 5775 | |
| | n | 6 | 11 | | 5 | 11 | | 5 | 9 | |

* Note

Unstim. = unstimulated
Diff. count = differential count

## 2:6 Summary Of Results Of Section 3:7

Table 2:6(i)

Results For homogonic S.ratti

| Week no. | Date Rats Infected | Total no. L3 Recovered | No. L3/gm. Faeces | Estimated No. L3 which theoretically could be produced from all faeces collected | % Non-Motile |
|---|---|---|---|---|---|
| YEAR 1 1983-1984 | | | | | |
| 1 | 15-8-83 | 60670 | 3315 | 60670 | - |
| 2 | 22-8-83 | - | - | - | - |
| 3 | 29-8-83 | 201700 | 10903 | 201700 | 14.8 |
| 4 | 5-9-83 | 79000 | 4158 | 90226 | - |
| 5 | 12-9-83 | 25000 | 1225 | 32966 | 7.4 |
| 6 | 19-9-83 | 3125 | 144 | 4472 | 3.4 |
| 7 | 26-9-83 | 58000 | 2566 | 91619 | 14.9 |
| 8 | 3-10-83 | 6000 | 256 | 7256 | 5.3 |
| 9 | 10-10-83 | 33000 | 1284 | 36724 | 14.7 |
| 10 | 17-10-83 | 4833 | 241 | 4833 | 0.0 |
| 11 | 24-10-83 | 25840 | 1298 | 25840 | 5.0 |
| 12 | 31-10-83 | 4667 | 200 | 4667 | 0.0 |
| 13 | 7-11-83 | 62000 | 3229 | 62000 | 10.1 |
| 14 | 14-11-83 | 21670 | 1223 | 21670 | 0.7 |
| 15 | 21-11-83 | 42670 | 1855 | 42670 | 0.0 |
| 16 | 28-11-83 | 3670 | 232 | 3670 | 12.0 |
| 17 | 5-12-83 | 64830 | 3325 | 81453 | 26.0 |
| 18 | 12-12-83 | 19500 | 990 | 24251 | 1.7 |
| 19 | 19-12-83 | - | - | - | - |
| 20 | 26-12-83 | - | - | - | - |
| 21 | 2-1-84 | - | - | - | - |
| 22 | 9-1-84 | 7905 | 462 | 7905 | 9.3 |
| 23 | 16-1-84 | 5000 | 394 | 5000 | 0.0 |
| 24 | 23-1=84 | 9900 | 550 | 9900 | 0.0 |
| 25 | 30-1-84 | 12677 | 689 | 20669 | 7.6 |
| 26 | 6-2-84 | 69670 | 3537 | 106450 | 3.5 |
| 27 | 13-2-84 | 25998 | 1870 | 25998 | 16.0 |
| 28 | 20-2-84 | 95400 | 5747 | 95400 | 2.5 |
| 29 | 27-2-84 | 21650 | 2303 | 21650 | 6.4 |
| 30 | 5-3-84 | 4902 | 299 | 9535 | 3.9 |
| 31 | 12-3-84 | 16170 | 893 | 16170 | 5.8 |
| 32 | 19-3-84 | - | - | - | - |
| 33 | 26-3-84 | 68830 | 3845 | 81904 | 5.1 |
| 34 | 2-4-84 | 47670 | 2563 | 65364 | 18.3 |
| 35 | 9-2-84 | 23170 | 1363 | 28758 | 1.4 |
| 36 | 16-4-84 | 33500 | 4085 | 33500 | 0.9 |
| 37 | 23-4-84 | - | - | - | - |
| 38 | 30-4-84 | 36500 | 2310 | 36500 | 1.8 |
| 39 | 7-5-84 | 44331 | 3534 | 59592 | 6.4 |
| 40 | 14-5-84 | 48330 | 2860 | 93800 | 13.4 |
| 41 | 21-5-84 | 35330 | 2091 | 53309 | 5.7 |
| 42 | 28-5-84 | 27915 | 1813 | 54561 | 0.6 |
| 43 | 4-6-84 | 135000 | 14835 | 135000 | 23.3 |
| 44 | 11-6-84 | 31300 | 1910 | 54063 | 6.0 |

| | | | | | |
|---|---|---|---|---|---|
| 45 | 18-6-84 | 23680 | 1894 | 28984 | 1.8 |
| 46 | 25-6-84 | 33336 | 3788 | 54174 | 0.0 |
| 47 | 2-7-84 | 31370 | 2070 | 31370 | 11.2 |
| 48 | 9-7-84 | 56700 | 5611 | 56700 | 6.3 |
| 49 | 16-7-84 | - | - | - | - |
| 50 | 23-7-84 | - | - | - | - |
| 51 | 30-7-84 | - | - | - | - |
| 52 | 6-8-84 | | | | |

YEAR 2 1984-1985

| | | | | | |
|---|---|---|---|---|---|
| 1 | 13-8-84 | - | - | - | - |
| 2 | 20-8-84 | 8869 | 785 | 25665 | 0.0 |
| 3 | 27-8-84 | 6300 | 643 | 14657 | 0.0 |
| 4 | 3-9-84 | 41250 | 3198 | 103605 | 3.8 |
| 5 | 10-9-84 | 5070 | 367 | 9773 | 2.5 |
| 6 | 17-9-84 | 43830 | 3879 | 98133 | 11.3 |
| 7 | 24-9-84 | 17900 | 1642 | 26768 | 0.0 |
| 8 | 1-10-84 | 118500 | 10128 | 162051 | 9.0 |
| 9 | 8-10-84 | 11500 | 855 | 11500 | 21.0 |
| 10 | 15-10-84 | 22050 | 1542 | 27138 | 23.0 |
| 11 | 22-10-84 | - | - | - | - |
| 12 | 29-10-84 | 10536 | 667 | 17004 | 0.0 |
| 13 | 5-11-84 | 22264 | 1700 | 25663 | 1.0 |
| 14 | 12-11-84 | - | - | - | - |
| 15 | 19-11-84 | - | - | - | - |
| 16 | 26-11-84 | - | - | - | - |
| 17 | 3-12-84 | 5719 | 397 | 9254 | 3.9 |
| 18 | 10-12-84 | - | - | - | - |
| 19 | 17-12-84 | - | - | - | - |
| 20 | 24-12-84 | - | - | - | - |
| 21 | 31-12-84 | - | - | - | - |
| 22 | 7-1-85 | 52000 | 3314 | 60719 | 4.9 |
| 23 | 14-1-85 | 54000 | 3064 | 81307 | 6.0 |
| 24 | 21-1-85 | - | - | - | - |
| 25 | 28-1-85 | 26264 | 1811 | 30611 | 4.0 |
| 26 | 4-2-85 | 29064 | 2004 | 33073 | 0.0 |
| 27 | 11-2-84 | 60631 | 4394 | 60631 | 15.0 |
| 28 | 18-2-85 | 15000 | 2000 | 15000 | 0.0 |
| 29 | 25-2-85 | 36670 | 3006 | 50496 | 14.4 |
| 30 | 4-3-85 | 36730 | 2340 | 36730 | 22.7 |
| 31 | 11-3-85 | 35330 | 2236 | 35330 | 21.5 |
| 32 | 18-3-85 | - | - | - | - |
| 33 | 25-3-85 | - | - | - | - |
| 34 | 1-4-85 | 8598 | 632 | 8598 | 0.0 |
| 35 | 8-4-85 | - | - | - | - |
| 36 | 15-4-85 | 18900 | 1196 | 18900 | 6.0 |
| 37 | 22-4-85 | 42704 | 1906 | 42704 | 14.0 |
| 38 | 29-4-85 | 46800 | 2218 | 46800 | 5.4 |
| 39 | 6-5-85 | 1000 | 68 | 1156 | 3.0 |
| 40 | 13-5-85 | 26000 | 1421 | 26000 | 12.2 |
| 41 | 20-5-85 | 3068 | 323 | 3068 | 2.5 |
| 42 | 27-5-85 | 27799 | 1418 | 32621 | 2.1 |
| 43 | 3-6-85 | 18396 | 1057 | 20616 | 11.5 |
| 44 | 10-6-85 | 26670 | 1657 | 26670 | 29.0 |
| 45 | 17-6-85 | 26136 | 1098 | 26136 | 0.0 |
| 46 | 24-6-85 | - | - | - | - |
| 47 | 1-7-85 | - | - | - | - |
| 48 | 8-7-85 | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| 49 | 15-7-85 | 54396 | 3073 | 54396 | 9.3 |
| 50 | 22-7-85 | 10268 | 723 | 10268 | 0.0 |
| 51 | 29-7-85 | - | - | - | - |
| 52 | 5-8-85 | - | - | - | - |

Table 2:6(ii)

Results For heterogonic S.ratti

| Week No. | Total No. L3 Recovered | No. L3/Gm. Faeces | Estimated No. L3 which theoretically could be produced from all faeces collected | % Non-Motile |
|---|---|---|---|---|
| YEAR 1 1983-1984 | | | | |
| 1 | 640 | 36 | 828 | 8.0 |
| 2 | - | - | - | - |
| 3 | 25800 | 2505 | 25800 | 11.9 |
| 4 | 13170 | 766 | 17917 | 1.3 |
| 5 | 2000 | 110 | 2670 | 7.4 |
| 6 | 11563 | 480 | 14154 | 2.7 |
| 7 | - | - | - | - |
| 8 | 7762 | 317 | 9346 | 21.2 |
| 9 | 8170 | 339 | 10001 | 21.2 |
| 10 | 4500 | 210 | 5788 | 50.0 |
| 11 | 4600 | 245 | 6677 | 50.0 |
| 12 | 13325 | 595 | 13325 | 3.2 |
| 13 | 4800 | 208 | 4800 | 2.0 |
| 14 | 6175 | 330 | 6175 | 20.4 |
| 15 | 20670 | 975 | 20670 | 3.8 |
| 16 | 10925 | 9518 | 12323 | 6.6 |
| 17 | 15170 | 712 | 19657 | 6.2 |
| 18 | - | - | - | - |
| 19 | - | - | - | - |
| 20 | - | - | - | - |
| 21 | - | - | - | - |
| 22 | 5225 | 307 | 5225 | 1.8 |
| 23 | 9668 | 424 | 14417 | 0.0 |
| 24 | 16775 | 937 | 21836 | 4.2 |
| 25 | 6782 | 6315 | 8990 | 2.7 |
| 26 | - | - | - | - |
| 27 | 6438 | 562 | 6438 | 4.9 |
| 28 | 6932 | 381 | 6932 | 3.7 |
| 29 | 14898 | 645 | 20057 | 6.3 |
| 30 | 13650 | 794 | 17618 | 7.4 |
| 31 | 5833 | 310 | 6857 | 0.0 |
| 32 | - | - | - | - |
| 33 | 13500 | 754 | 13500 | 2.4 |
| 34 | 25830 | 1510 | 43503 | 21.3 |
| 35 | 8000 | 575 | 10008 | 18.6 |
| 36 | 13064 | 1306 | 13064 | 5.8 |
| 37 | 8335 | 583 | 18127 | 6.6 |
| 38 | 15670 | 1178 | 16613 | 2.1 |
| 39 | 32170 | 2401 | 35051 | 8.4 |
| 40 | 17225 | 1083 | 39000 | 15.0 |
| 41 | - | - | - | - |
| 42 | 16790 | 1235 | 35307 | 8.8 |
| 43 | 3600 | 316 | 3600 | 2.3 |
| 44 | 16800 | 1209 | 36740 | 2.7 |
| 45 | 15631 | 1797 | 15631 | 19.8 |
| 46 | 7098 | 703 | 12017 | 0.0 |

| | | | | |
|---|---|---|---|---|
| 47 | 7096 | 1223 | 7096 | 0.0 |
| 48 | 5732 | 630 | 5732 | 0.0 |
| 49 | - | - | - | - |
| 50 | - | - | - | - |
| 51 | - | - | - | - |
| 52 | - | - | - | - |

YEAR 2 1984-1985

| | | | | |
|---|---|---|---|---|
| 1 | 5367 | 511 | 5367 | 0.0 |
| 2 | 0 | 0 | | |
| 3 | 28025 | 2260 | 56502 | 0.0 |
| 4 | 3198 | 305 | 7736 | 0.0 |
| 5 | 8500 | 696 | 15442 | 1.9 |
| 6 | 6300 | 594 | 14969 | 0.0 |
| 7 | 1750 | 173 | 3327 | 5.0 |
| 8 | 17025 | 2702 | 24051 | 3.5 |
| 9 | - | - | - | - |
| 10 | 31330 | 2589 | 31330 | 0.0 |
| 11 | - | - | - | - |
| 12 | 4550 | 251 | 3570 | 19.0 |
| 13 | 0 | 0 | | |
| 14 | - | - | - | - |
| 15 | 12800 | 1143 | 21257 | 0.0 |
| 16 | - | - | - | - |
| 17 | - | - | - | - |
| 18 | 9149 | 257 | 9149 | 2.4 |
| 19 | - | - | - | - |
| 20 | - | - | - | - |
| 21 | - | - | - | - |
| 22 | - | - | - | - |
| 23 | 1000 | 76 | 3412 | 10.0 |
| 24 | - | - | - | - |
| 25 | 13080 | 998 | 26460 | 0.0 |
| 26 | 0 | 0 | | |
| 27 | 30133 | 1826 | 48578 | 0.0 |
| 28 | 6332 | 1213 | 6332 | 0.0 |
| 29 | 95000 | 5864 | 106780 | 0.0 |
| 30 | 5670 | 332 | 5670 | 0.0 |
| 31 | 35000 | 2652 | 35000 | 0.0 |
| 32 | - | - | - | - |
| 33 | - | - | - | - |
| 34 | 36000 | 2083 | 36000 | 12.0 |
| 35 | 1000 | 61 | 1000 | 1.0 |
| 36 | 45330 | 3170 | 45330 | 0.0 |
| 37 | 15812 | 742 | 19895 | 0.0 |
| 38 | 46800 | 2463 | 46800 | 0.0 |
| 39 | - | - | - | - |
| 40 | 28800 | 1565 | 39757 | 1.8 |
| 41 | 15000 | 1456 | 15000 | 8.2 |
| 42 | 10330 | 733 | 10330 | 0.0 |
| 43 | 39330 | 2537 | 39330 | 0.0 |
| 44 | 30000 | 2326 | 30000 | 2.0 |
| 45 | 46379 | 2710 | 46379 | 1.0 |
| 46 | 53660 | 3650 | 53660 | 0.0 |
| 47 | - | - | - | - |
| 48 | - | - | - | - |
| 49 | 31330 | 1958 | 39163 | 5.1 |
| 50 | 4835 | 361 | 4835 | 5.0 |
| 51 | - | - | - | - |

The week numbers refer to the same dates shown in Table 2:6(i)

Table 2:6(iii)

Results For S.venezuelensis

| Week No. | Date Rats Infected | Total No. L3 Recovered | No.L3/Gm. Faeces | Estimated No. L3 which theoretically could be produced from all faeces collected | % Non-Motile |
|---|---|---|---|---|---|
| YEAR 1 1983-1984 | | | | | |
| 1 | 26-8-83 | 43670 | 3580 | 43670 | 11.2 |
| 2 | 2-9-83 | - | - | - | - |
| 3 | 9-9-83 | 5670 | 336 | 5670 | 5.6 |
| 4 | 16-9-83 | 15170 | 632 | 17066 | 6.0 |
| 5 | 23-9-83 | 81000 | 8265 | 81000 | 22.4 |
| 6 | 30-9-83 | 12170 | 1512 | 12170 | 17.3 |
| 7 | 7-10-83 | 2670 | 102 | 2670 | 30.5 |
| 8 | 14-10-83 | 1025 | 58 | 1025 | 4.1 |
| 9 | 21-10-83 | 75500 | 6235 | 75500 | 27.6 |
| 10 | 28-10-83 | 400 | 26 | 400 | 27.3 |
| 11 | 4-11-83 | 7761 | 342 | 7761 | 49.3 |
| 12 | 11-11-83 | 1650 | 146 | 1650 | 50.0 |
| 13 | 18-11-83 | 8883 | 584 | 8883 | 48.8 |
| 14 | 25-11-83 | 400 | 48 | 400 | 0.0 |
| 15 | 2-12-83 | 4000 | 131 | 4000 | 22.6 |
| 16 | 9-12-83 | 15932 | 1099 | 15932 | 5.0 |
| 17 | 16-12-83 | - | - | - | - |
| 18 | 23-12-83 | - | - | - | - |
| 19 | 30-12-83 | - | - | - | - |
| 20 | 6-1-84 | 9972 | 669 | 9972 | 9.3 |
| 21 | 13-1-84 | 32830 | 2027 | 32830 | 33.4 |
| 22 | 20-1-84 | 11670 | 627 | 11670 | 1.4 |
| 23 | 27-1-84 | 6300 | 325 | 11334 | 35.7 |
| 24 | 3-2-84 | 0 | 0 | | |
| 25 | 10-2-84 | - | - | - | - |
| 26 | 17-2-84 | 0 | 0 | | |
| 27 | 24-2-84 | 7534 | 638 | 7534 | 6.5 |
| 28 | 2-3-84 | 80000 | 5556 | 80000 | 6.6 |
| 29 | 9-3-84 | 19750 | 1056 | 29150 | 50.0 |
| 30 | 16-3-84 | 33830 | 1911 | 60588 | 26.7 |
| 31 | 23-3-84 | 33857 | 1743 | 35923 | 2.9 |
| 32 | 30-3-84 | 2060 | 121 | 2872 | 13.4 |
| 33 | 6-4-84 | 18932 | 1372 | 18932 | 33.8 |
| 34 | 13-4-84 | 14500 | 868 | 15455 | 2.5 |
| 35 | 20-4-84 | - | - | - | - |
| 36 | 27-4-84 | - | - | - | - |
| 37 | 4-5-84 | 32704 | 2031 | 36360 | 5.2 |
| 38 | 11-5-84 | - | - | - | - |
| 39 | 18-5-84 | 115375 | 6631 | 145876 | 15.0 |
| 40 | 25-5-84 | 12532 | 915 | 18752 | 10.0 |
| 41 | 1-6-84 | 91800 | 9663 | 91800 | 13.1 |
| 42 | 8-6-84 | 25330 | 2093 | 41651 | 2.6 |
| 43 | 15-6-84 | 8085 | 735 | 8600 | 3.0 |
| 44 | 22-6-84 | 10540 | 811 | 12243 | 16.8 |
| 45 | 29-6-84 | - | - | - | - |
| 46 | 6-7-84 | 6915 | 1032 | 6915 | 9.8 |

| | | | | | |
|---|---|---|---|---|---|
| 47 | 13-7-84 | 5 | | | |
| 48 | 20-7-84 | - | - | - | - |
| 49 | 27-7-84 | - | - | - | - |
| 50 | 3-8-84 | - | - | - | - |
| 51 | 10-8-84 | - | - | - | - |
| 52 | 17-8-84 | 9332 | 735 | 18370 | 5.0 |

YEAR 2 1984-1985

| | | | | | |
|---|---|---|---|---|---|
| 1 | 24-8-84 | 41800 | 6967 | 41800 | 1.3 |
| 2 | 31-8-84 | - | - | - | - |
| 3 | 7-9-84 | 5831 | 435 | 11923 | 0.0 |
| 4 | 14-9-84 | 11328 | 1287 | 24072 | 2.3 |
| 5 | 21-9-84 | 16128 | 3934 | 16128 | 0.0 |
| 6 | 28-9-84 | 40330 | 4859 | 52963 | 6.0 |
| 7 | 5-10-84 | 12652 | 1278 | 16869 | 22.0 |
| 8 | 12-10-84 | - | - | - | - |
| 9 | 19-10-84 | 28280 | 1964 | 52239 | 7.1 |
| 10 | 26-10-84 | 16500 | 6346 | 16500 | 10.8 |
| 11 | 2-11-84 | 9830 | 806 | 19660 | 20.0 |
| 12 | 9-11-84 | 30500 | 2500 | 45750 | 50.0 |
| 13 | 16-11-84 | - | - | - | - |
| 14 | 23-11-84 | - | - | - | - |
| 15 | 30-11-84 | 14123 | 1130 | 17738 | 4.2 |
| 16 | 7-12-84 | - | - | - | - |
| 17 | 14-12-84 | - | - | - | - |
| 18 | 21-12-84 | - | - | - | - |
| 19 | 28-12-84 | - | - | - | - |
| 20 | 4-1-85 | - | - | - | - |
| 21 | 11-1-85 | 15867 | 912 | 23983 | 16.7 |
| 22 | 18-1-85 | - | - | - | - |
| 23 | 25-1-85 | 8169 | 529 | 9359 | 3.5 |
| 24 | 1-2-85 | 11250 | 787 | 11250 | 6.3 |
| 25 | 8-2-85 | - | - | - | - |
| 26 | 15-2-85 | 15950 | 1173 | 16888 | 0.0 |
| 27 | 22-2-85 | 8633 | 3197 | 8633 | 60.0 |
| 28 | 1-3-85 | - | - | - | - |
| 29 | 8-3-85 | 88000 | 5207 | 88000 | 12.0 |
| 30 | 15-3-85 | - | - | - | - |
| 31 | 22-3-85 | - | - | - | - |
| 32 | 29-3-85 | - | - | - | - |
| 33 | 5-4-85 | - | - | - | - |
| 34 | 12-4-85 | - | - | - | - |
| 35 | 19-4-85 | - | - | - | - |
| 36 | 26-4-85 | 35000 | 1913 | 35000 | 5.4 |
| 37 | 3-5-85 | 32704 | 1869 | 37189 | 6.0 |
| 38 | 10-5-85 | 31500 | 1898 | 31500 | 11.9 |
| 39 | 17-5-85 | 31330 | 2034 | 37026 | 4.1 |
| 40 | 24-5-85 | 10330 | 684 | 10330 | 0.0 |
| 41 | 31-5-84 | - | - | - | - |
| 42 | 7-6-85 | 1333 | 106 | 1333 | 1.0 |
| 43 | 14-6-85 | 1666 | 85 | 1905 | 5.0 |
| 44 | 21-6-85 | - | - | - | - |
| 45 | 28-6-85 | - | - | - | - |
| 46 | 5-7-85 | - | - | - | - |
| 47 | 12-7-85 | - | - | - | - |
| 48 | 19-7-85 | 12503 | 817 | 19694 | 4.0 |
| 49 | 26-7-85 | - | - | - | - |
| 50 | 2-8-85 | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| 51 | 9-8-85 | - | - | - | - |
| 52 | 16-8-85 | - | - | - | - |

Fig.2:1

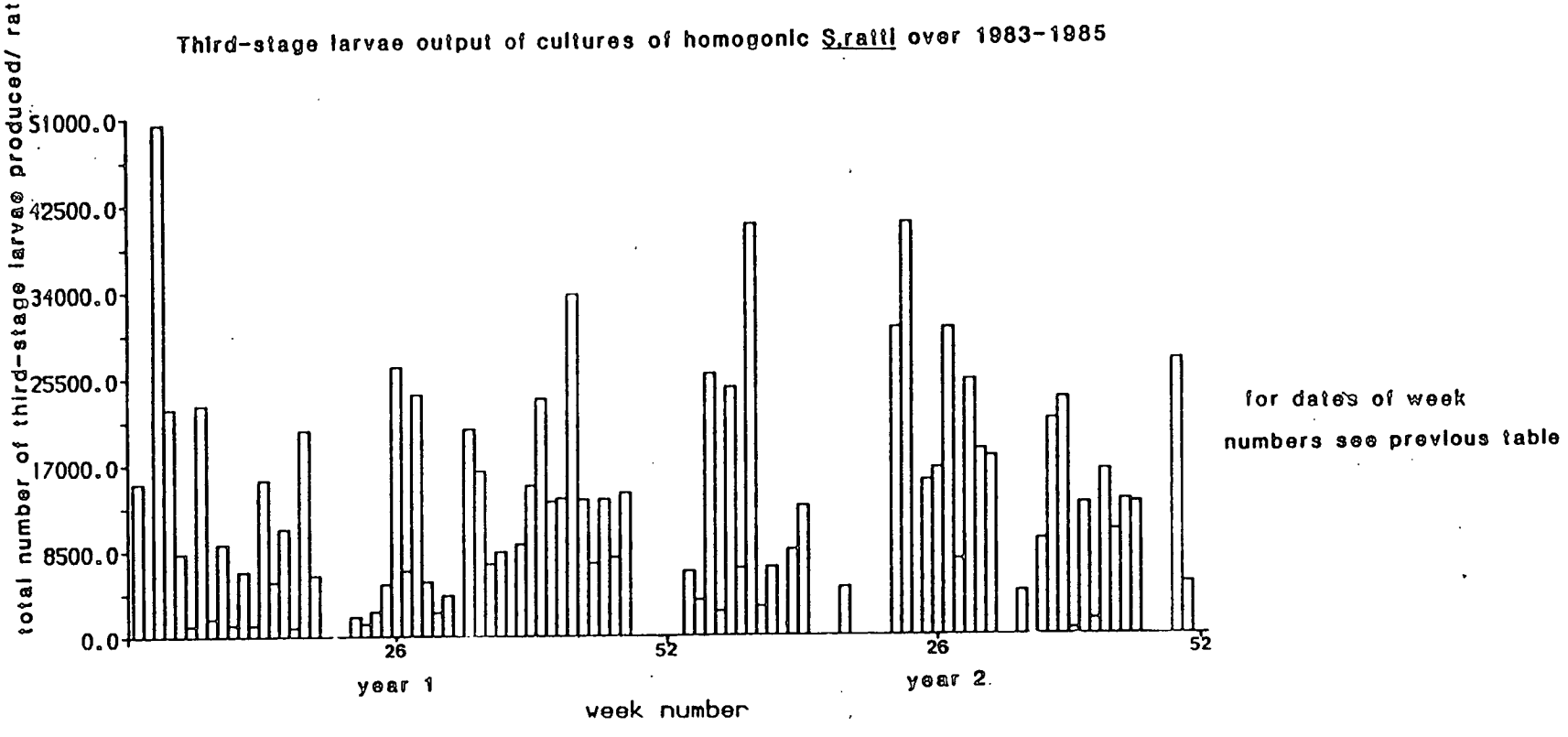

Third-stage larvae output of cultures of homogonic S.ratti over 1983-1985
total number of third-stage larvae produced/ rat
51000.0
42500.0
34000.0
25500.0
17000.0
8500.0
0.0
26
52
26
52
year 1
year 2
week number
for dates of week numbers see previous table

Fig.2:2

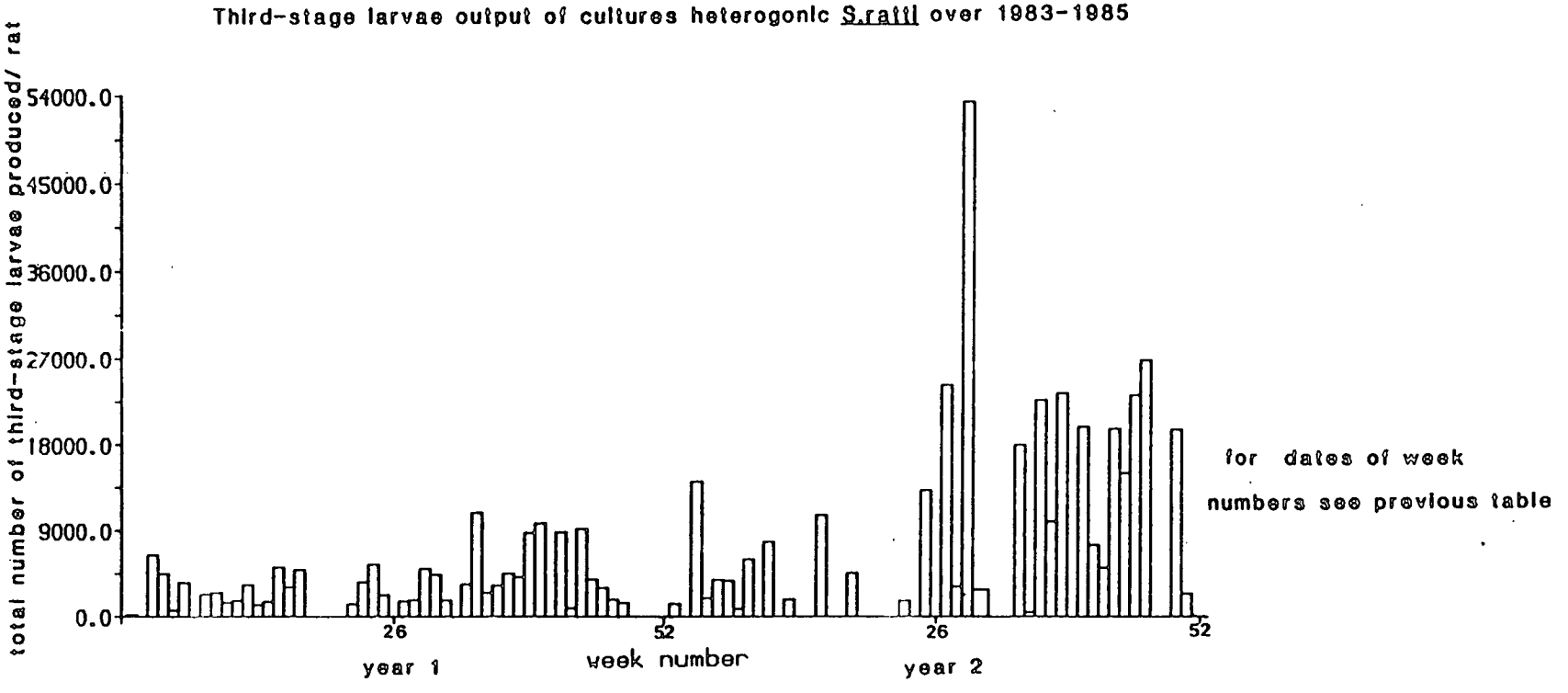
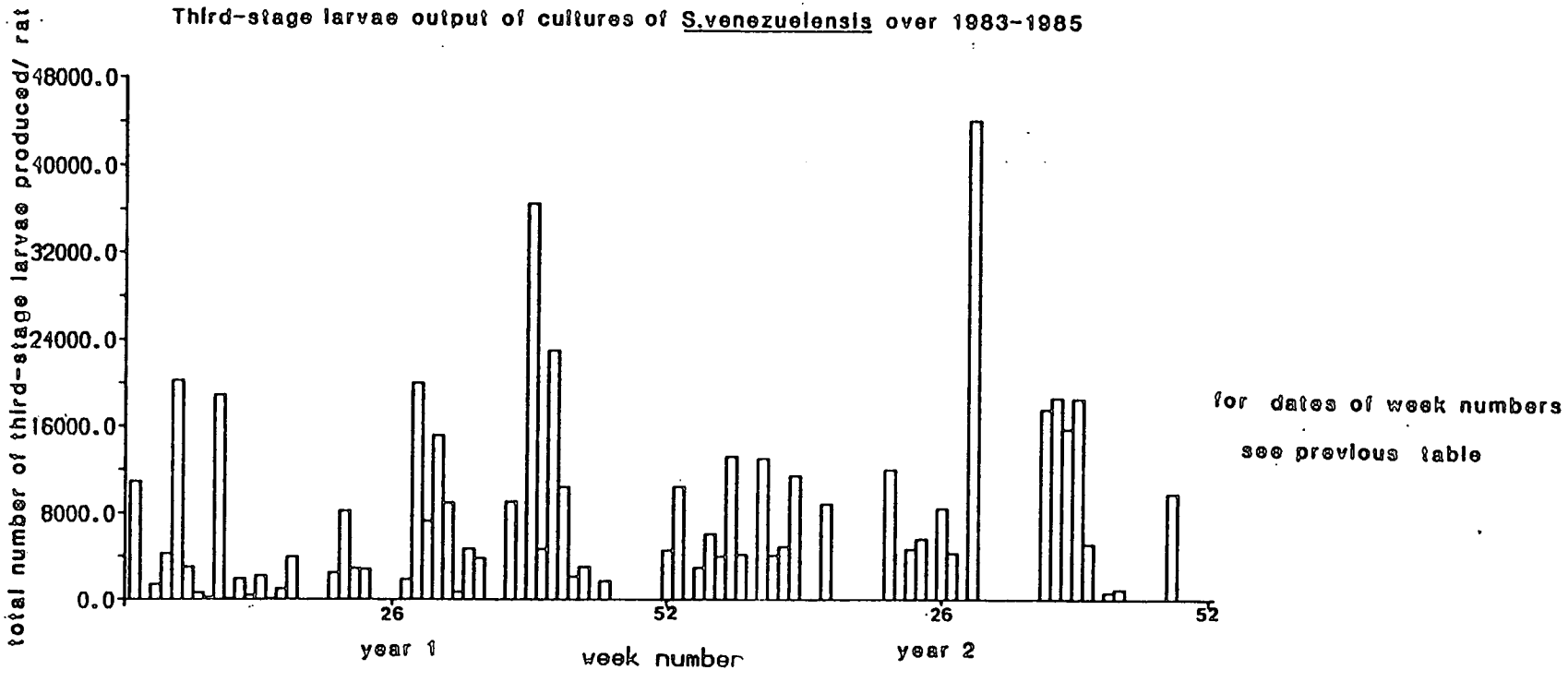

Third-stage larvae output of cultures heterogonic S.ratti over 1983-1985
total number of third-stage larvae produced/ rat
54000.0
45000.0
36000.0
27000.0
18000.0
9000.0
0.0
26
52
26
52
year 1
week number
year 2
for dates of week numbers see previous table

Fig.2:3


Third-stage larvae output of cultures of S.venezuelensis over 1983-1985
total number of third-stage larvae produced/ rat
48000.0
40000.0
32000.0
24000.0
16000.0
8000.0
0.0
26
52
26
52
year 1
week number
year 2
for dates of week numbers
see previous table

## 2:7 Statistical analysis of results from Section 3:8

Table 2:7(i)

Egg Data

| Expt | Treatment | Mean No. Eggs in Utero/Worm /Rat | Total No. Worms Assayed /Treatment | Correlation Coefficient | P Value |
|---|---|---|---|---|---|
| Expt 3 Chapter 4 | Day 8 heterogonic *S.ratti* Controls | 6.000<br>5.500<br>5.700<br>5.400<br>5.200<br>5.250 | 120 | 0.998 | 0.01 |
| Expt 5 Chapter 5 | Day 8 heterogonic *S.ratti* Priming Controls | 6.007<br>6.500<br>6.143<br>6.800 | 54 | 0.998 | 0.01 |
| | Day 8 homogonic *S.ratti* Challenge Controls | 6.214<br>6.923<br>6.455<br>6.667<br>6.750 | 66 | 0.995 | 0.01 |
| Expt 5 Chapter 8 | Day 8 homogonic *S.ratti* Challenge Controls | 7.277<br>7.421<br>7.200<br>7.000 | 65 | 0.995 | 0.01 |
| | Day 16 homogonic *S.ratti* Challenge Controls | 7.200<br>7.200<br>7.200<br>7.600 | 80 | 0.989 | 0.05 |
| Expt 6 Chapter 8 | Day 8 heterogonic *S.ratti* Priming Controls | 6.588<br>6.111<br>6.143 | 43 | 0.999 | 0.01 |
| | Day 8 heterogonic *S.ratti* Challenge Controls | 6.875<br>5.900<br>5.941<br>6.500 | 67 | 0.978 | 0.05 |

Table 2:7(ii)

Lymphocyte transformation data

| Expt | Treatment | | Mean Differential Count | n | Correlation Coefficient | P Value |
|---|---|---|---|---|---|---|
| Expt 9 Chapter 3 | Rat 1 | 5% Serum | 9.791 | 10 | 0.989 | 0.01 |
| | | 10% Serum | 11.486 | 11 | 0.791 | ) 0.10 |
| | | 15% Serum | 11.477 | 11 | 0.641 | ) 0.10 |
| | Rat 2 | 5% Serum | 10.028 | 11 | 0.981 | 0.01 |
| | | 10% Serum | 11.164 | 12 | 0.982 | 0.01 |
| | | 15% Serum | 11.501 | 11 | 0.963 | 0.01 |
| | Rat 3 | 5% Serum | 10.340 | 12 | 0.966 | 0.01 |
| | | 10% Serum | 9.815 | 12 | 0.925 | 0.05 |
| | | 15% Serum | 10.375 | 11 | 0.923 | 0.05 |
| Overall | 15% Serum Controls | | 5.480<br>5.775<br>5.323 | 18 | 0.975 | 0.01 |

3:1Summary Of Results

Table 3:1(i)

The proportion of the homogonic S.ratti dose recovered over the course of infection in Expt 1

| Day post-infection | Mean proportion of the dose recovered | n | SD |
|---|---|---|---|
| 8 | 0.538 | 6 | 0.102 |
| 14 | 0.349 | 6 | 0.093 |
| 20 | 0.224 | 6 | 0.114 |
| 26 | 0.073 | 6 | 0.068 |
| 32 | 0.014 | 8 | 0.023 |
| mean exact dose at priming | 97.5 | | |

Table 3:1(ii)

The proportion of the homogonic S.ratti dose recovered over the course of infection in Expt 2

| Day post-infection | Mean proportion of the dose recovered | n | SD | Mean no. of eggs in utero/ worm | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| 8 (controls) | 0.726 | 6 | 0.165 | 7.817 | 120 | 1.361 | 7.908 | 6 | 0.591 |
| 8 | 0.703 | 6 | 0.090 | 7.908 | 120 | 1.595 | 7.817 | 6 | 0.380 |
| 14 | 0.459 | 6 | 0.084 | 5.683 | 120 | 1.243 | 5.683 | 6 | 0.342 |
| 20 | 0.234 | 6 | 0.095 | 4.547 | 86 | 1.752 | 4.413 | 6 | 0.869 |
| 26 | 0.079 | 6 | 0.043 | 1.977 | 43 | 1.928 | 2.202 | 6 | 1.301 |
| 32 | 0.045 | 6 | 0.050 | 2.391 | 23 | 2.488 | 2.197 | 4 | 2.356 |
| mean exact dose | subcutaneous injection | | | | | 89.0 | | | |
| | skin-application | | | | | 92.5 | | | |

Table 3:1(iii)

The proportion of the heterogonic S.ratti dose recovered over the course of infection in Expt 3

| Day post-infection | Mean proportion of the dose recovered | n | SD | Mean no. of eggs in utero/ worm | n | SD | Mean no. of eggs in utero/ worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| 8 (controls) | 0.445 | 6 | 0.320 | 5.962 | 78 | 1.55 | 5.800 | 6 | 0.918 |
| 8 | 0.681 | 6 | 0.101 | 5.508 | 120 | 1.52 | 5.508 | 6 | 0.301 |
| 14 | 0.353 | 6 | 0.046 | 3.576 | 118 | 1.73 | 3.590 | 6 | 0.636 |
| 20 | 0.080 | 6 | 0.047 | 2.227 | 44 | 1.55 | 1.983 | 6 | 0.788 |
| 26 | 0.007 | 6 | 0.009 | 3.000 | 4 | 1.15 | 3.000 | 3 | 1.000 |
| 32 | 0.007 | 6 | 0.012 | 2.250 | 4 | 1.26 | 2.165 | 2 | 0.233 |
| mean exact dose | subcutaneous injection | | | | | | 95.2 | | |
| | skin-application | | | | | | 95.1 | | |

Table 3:1(iv)

Proportion of the S.venezuelensis dose recovered on day 8 post-infection in Expt 4

| Treatment | Mean proportion of the dose recovered | n | SD | mean exact dose |
|---|---|---|---|---|
| subcutaneous injection | 0.056 | 4 | 0.066 | 94.8 |
| skin-application | 0.020 | 4 | 0.008 | 97.8 |

3:2 Distribution data

Table 3:2(i)

Distribution of adult homogonic S.ratti worms along the intestine in Expt. 2

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| Day 8 | Mean | 0.41 | 0.32 | 0.16 | 0.07 | 0.02 | 0.002 | 0.00 | 0.00 | 6 |
| | SD | 0.04 | 0.06 | 0.04 | 0.04 | 0.02 | 0.004 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 65 | | | | | |
| Day 8 (controls) | Mean | 0.40 | 0.36 | 0.16 | 0.06 | 0.03 | 0.01 | 0.00 | 0.00 | 6 |
| | SD | 0.13 | 0.06 | 0.07 | 0.04 | 0.03 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 65 | | | | | |
| Day 14 | Mean | 0.45 | 0.29 | 0.14 | 0.10 | 0.01 | 0.01 | 0.00 | 0.01 | 6 |
| | SD | 0.06 | 0.14 | 0.06 | 0.08 | 0.01 | 0.03 | 0.00 | 0.02 | |
| | mean no. of worms per rat | | | | 42 | | | | | |
| Day 20 | Mean | 0.45 | 0.27 | 0.16 | 0.09 | 0.02 | 0.01 | 0.00 | 0.00 | 6 |
| | SD | 0.08 | 0.09 | 0.06 | 0.07 | 0.04 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 22 | | | | | |
| Day 26 | Mean | 0.29 | 0.41 | 0.17 | 0.01 | 0.04 | 0.07 | 0.00 | 0.02 | 6 |
| | SD | 0.27 | 0.31 | 0.20 | 0.03 | 0.09 | 0.13 | 0.00 | 0.05 | |
| | mean no. of worms per rat | | | | 7 | | | | | |
| Day 32 | Mean | 0.32 | 0.24 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 4 |
| | SD | 0.47 | 0.28 | 0.32 | 0.00 | 0.00 | 0.00 | 0.00 | 0.15 | |
| | mean no. of worms per rat | | | | 4 | | | | | |

Table 3:2(ii)

Distribution of adult heterogonic S.ratti worms along the intestine in Expt 3

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| Day 8 | Mean | 0.26 | 0.44 | 0.23 | 0.03 | 0.02 | 0.01 | 0.002 | 0.00 | 6 |
| | SD | 0.13 | 0.17 | 0.09 | 0.03 | 0.02 | 0.02 | 0.004 | 0.00 | |
| | mean no. of worms per rat | | | | 65 | | | | | |
| Day 8 (controls) | Mean | 0.40 | 0.43 | 0.11 | 0.03 | 0.01 | 0.01 | 0.00 | 0.00 | 6 |
| | SD | 0.08 | 0.09 | 0.06 | 0.02 | 0.02 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 42 | | | | | |
| Day 14 | Mean | 0.35 | 0.37 | 0.18 | 0.07 | 0.02 | 0.01 | 0.00 | 0.01 | 6 |
| | SD | 0.08 | 0.08 | 0.06 | 0.07 | 0.03 | 0.01 | 0.00 | 0.02 | |
| | mean no. of worms per rat | | | | 34 | | | | | |
| Day 20 | Mean | 0.25 | 0.10 | 0.03 | 0.06 | 0.08 | 0.04 | 0.05 | 0.40 | 6 |
| | SD | 0.16 | 0.09 | 0.05 | 0.10 | 0.12 | 0.06 | 0.08 | 0.35 | |
| | mean no. of worms per rat | | | | 8 | | | | | |
| Day 26 | Mean | 0.17 | 0.17 | 0.00 | 0.67 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.29 | 0.29 | 0.00 | 0.58 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| Day 32 | Mean | 0.00 | 0.00 | 0.34 | 0.67 | 0.00 | 0.00 | 0.00 | 0.00 | 2 |
| | SD | 0.00 | 0.00 | 0.47 | 0.47 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |

Table 3:2(iii)

Distribution of adult S.venezuelensis along the intestine in Expt 4

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| sub-cutaneous injection | Mean | 0.57 | 0.43 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.51 | 0.53 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | | 5 | | | | |
| skin-application | Mean | 0.67 | 0.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.47 | 0.47 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | | 2 | | | | |

3:3 Statistical analysis of data

The results of the analyses carried out on data in Chapter 4 are given as an example of the type of tests used to analyse data throughout this study.

3:3a Worm data

Table 3:3a(i)

Single classification anova on data in Expt 1

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 4 | 7847.296 | 1961.824 | 43.37 |
| Within | 27 | 1221.267 | 45.232 | |
| Total | | 9068.563 | | |

$F_{(4,27)} = 6.33$ 0.1%

Table 3:3a(ii)

Single classification anova on data in Expt 1, days 8, 14 and 20 only

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 2 | 1188.598 | 594.299 | 11.96 |
| Within | 15 | 745.700 | 49.713 | |
| Total | | 1934.298 | | |

$F_{(2,15)} = 11.96$ 0.1%

Table 3:3a(iii)

Single classification anova on data in Expt 2, days 8, 14 and 20 only

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 3 | 3698.367 | 1232.789 | 20.17 |
| Within | 20 | 1222.700 | 61.135 | |
| Total | | 4921.068 | | |

$F_{(3,20)} = 8.10$ 0.1%

Table 3:3a(iv)

Single classification anova on data in Expt 3, days 8, 14 and 20 only

| Source Of Variation | df | SS | MS | $F_o$ |
|---|---|---|---|---|
| Among Gps. | 3 | 4981.801 | 1660.600 | 12.60 |
| Within | 20 | 2636.179 | 131.809 | |
| Total | | 7617.980 | | |

$F_{(3,20)} = \underline{8.10}$ 0.1%

Table 3:3a(v)

t-test comparing the two treatments in Expt 4

| Source Of Variation | df | SS | MS | t-value |
|---|---|---|---|---|
| Among Gps. | 1 | 15.554 | 15.554 | 0.55 |
| Within | 6 | 303.107 | 50.518 | |
| Total | | 318.661 | | |

$t_{(6)} = \underline{1.27}$ 25%

Table 3:3a(vi)

Comparison of the proportion of the dose recovered for controls in Expts 1, 2 and 3, using a single classification anova

| Source Of Variation | df | SS | MS | $F_o$ |
|---|---|---|---|---|
| Among Gps. | 4 | 1419.536 | 354.884 | 2.60 |
| Within | 25 | 3411.600 | 136.464 | |
| Total | | 4831.140 | | |

$F_{(4,25)} = \underline{2.76}$ 5%

Table 3:3a(vii)

Two-way anova comparing the proportion of the dose recovered on days 8, 14 and 20, in Expts 1, 2 and 3

| Source Of Variation | df | SS | MS | $F_o$ |
|---|---|---|---|---|
| Among Cols. | 2 | 8247.453 | 4123.727 | 85.24 |
| Among Rows | 2 | 586.031 | 293.726 | 6.06 |
| Interaction | 4 | 635.286 | 158.821 | 3.28 |
| Among Gps. | 8 | 9468.769 | 1183.596 | |
| Within | 45 | 2176.972 | 48.377 | |
| Total | | 11645.741 | | |

$F_{(4,45)} = \underline{3.10}$ 2.5%

Table 3:3a(viii)

Two-way anova comparing the proportion of the dose recovered on all days for Expts 1 and 2

| Source Of Variation | df | SS | MS | $F_o$ |
|---|---|---|---|---|
| Among Cols. | 4 | 16953.150 | 4238.288 | 78.70 |
| Among Rows | 1 | 454.889 | 454.889 | 8.45 |
| Interaction | 4 | 249.792 | 62.448 | 1.16 |
| Among Gps. | 9 | 17657.831 | 1961.981 | |
| Within | 50 | 2692.653 | 53.853 | |
| Total | | 20350.484 | | |

$F_{(4,50)} = \underline{5.60}$ 0.1% $F_{(1,50)} = \underline{7.15}$ 1% $F_{(4,50)} = \underline{2.57}$ 5%

b) Egg data

Table 3:3b(i)

Two-level nested anova on egg data in Expt 2

| Source Of Variation | df | SS | MS | $F_o$ |
|---|---|---|---|---|
| Among Gps. | 5 | 2020.775 | 404.155 | 40.87 |
| Among Subgps. | 28 | 276.891 | 9.889 | 4.78 |
| Within | 478 | 988.803 | 2.069 | |
| Total | 511 | 3286.469 | | |

$F_{(5,28)} = \underline{5.66}$ 0.1% $F_{(24,\infty)} = \underline{2.13}$

Table 3:3b(ii)

Single classification anova on egg data in Expt 2

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 5 | 178.227 | 35.645 | 32.16 |
| Within(Error) | 28 | 31.035 | 1.108 | |
| Total | | 209.261 | | |

$F_{(5,28)}$=5.66

Table 3:3b(iii)

Two-level nested anova on egg data in Expt 3

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 5 | 589.830 | 117.966 | 25.18 |
| Among Subgps. | 23 | 107.966 | 4.685 | 1.95 |
| Within(error) | 339 | 814.146 | 2.402 | |
| Total | 367 | 1511.728 | | |

$F_{(5,23)}$=6.08 0.1% $F_{(12,\infty)}$=1.75 5%

Table 3:3b(iv)

Single classification anova on egg data in Expt 3

| Source Of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 5 | 68.634 | 13.634 | 26.47 |
| Within | 23 | 11.847 | 0.515 | |
| Total | | 80.017 | | |

$F_{(5,23)}$=6.08 0.1%

## 4:1 Summary of results

Table 4:1(i)

Homologous challenge with homogonic S.ratti, Expt 1

| Treatment | Mean proportion of the dose recovered | | n | SD |
|---|---|---|---|---|
| homologous challenge Gp. A | 0.054 | | 12 | 0.056 |
| day 8 secondary skin-application controls, Gp.C2 | 0.546 | | 12 | 0.102 |
| day 8 priming injection controls, Gp.C1 | 0.539 | | 6 | 0.164 |
| day 8 secondary injection controls, Gp.C3 | 0.547 | | 6 | 0.138 |
| mean exact dose | priming | skin-application | | 97.08 |
| | | injection | | 95.17 |
| | challenge | skin-application | | 95.70 |
| | | injection | | 90.71 |

Table 4:1(ii)

Homologous challenge with homogonic S.ratti, Expts 1 and 2

| Treatment | Mean prop. of the dose recovered | n | SD | Mean no. of eggs in utero/ | n | SD | Mean no. of eggs in utero worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Expt 2 Gp. A1 | 0.065 | 10 | 0.114 | 2.776 | 47 | 1.658 | 2.358 | 4 | 0.917 |
| homologous challenge Expt 3 Gp. A2 | 0.031 | 11 | 0.065 | 1.516 | 31 | 1.264 | 1.325 | 6 | 0.429 |
| priming controls Expts 2 and 3, Gp.C1 | 0.500 | 5 | 0.237 | 8.607 | 83 | 1.585 | 8.624 | 5 | 0.414 |
| challenge controls Expt 2, Gp.C2 | 0.417 | 6 | 0.236 | 6.943 | 88 | 1.193 | 1.050 | 6 | 0.616 |
| challenge controls Expt 3, Gp.C3 | 0.632 | 5 | 0.396 | 7.770 | 74 | 1.641 | 7.782 | 4 | 0.289 |

| | | | |
|---|---|---|---|
| mean exact dose | priming Expts 2 and 3 | skin-application | 96.36 |
| | | injection | 96.20 |
| | challenge Expt 2 | skin-application | 96.73 |
| | | injection | 97.80 |
| | challenge Expt 3 | skin-application | 98.50 |
| | | injection | 95.67 |

4:2 Worm distribution data

Table 4:2(i)

Distribution of worms along the intestine in Expt 1

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp. A | Mean | 0.34 | 0.29 | 0.10 | 0.07 | 0.08 | 0.09 | 0.01 | 0.03 | 8 |
| | SD | 0.35 | 0.32 | 0.10 | 0.10 | 0.11 | 0.14 | 0.04 | 0.07 | |
| | mean no. of worms per rat | | | | | 5 | | | | |
| day 8 secondary controls Gp.C2 | Mean | 0.46 | 0.31 | 0.18 | 0.06 | 0.003 | 0.00 | 0.00 | 0.00 | 12 |
| | SD | 0.10 | 0.07 | 0.07 | 0.05 | 0.009 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | | 52 | | | | |
| day 8 priming controls Gp.C1 | Mean | 0.37 | 0.37 | 0.17 | 0.10 | 0.01 | 0.003 | 0.00 | 0.00 | 6 |
| | SD | 0.12 | 0.06 | 0.07 | 0.06 | 0.02 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | | 51 | | | | |
| day 8 challenge controls Gp.C3 | Mean | 0.46 | 0.32 | 0.14 | 0.07 | 0.01 | 0.01 | 0.00 | 0.00 | 6 |
| | SD | 0.12 | 0.08 | 0.07 | 0.04 | 0.02 | 0.02 | 0.00 | 0.00 | |
| | mean no. od worms per rat | | | | | 50 | | | | |

Table 4:2(ii)

Distribution of worms along the intestine in Expts 2 and 3

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Expt 2 Gp. A1 | Mean | 0.83 | 0.06 | 0.10 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 6 |
| | SD | 0.27 | 0.10 | 0.16 | 0.02 | 0.00 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | | 6 | | | | |
| homologous challenge Expt 3 Gp. A2 | Mean | 0.54 | 0.06 | 0.12 | 0.11 | 0.00 | 0.04 | 0.03 | 0.02 | 4 |
| | SD | 0.36 | 0.11 | 0.18 | 0.15 | 0.00 | 0.06 | 0.07 | 0.03 | |
| | mean no. of worms per rat | | | | | 3 | | | | |
| priming controls Gp.C1 | Mean | 0.46 | 0.34 | 0.10 | 0.06 | 0.03 | 0.004 | 0.00 | 0.004 | 5 |
| | SD | 0.17 | 0.09 | 0.08 | 0.03 | 0.02 | 0.01 | 0.00 | 0.01 | |
| | mean no. of worms per rat | | | | | 48 | | | | |
| challenge controls Expt 2 Gp.C2 | Mean | 0.36 | 0.42 | 0.17 | 0.04 | 0.004 | 0.002 | 0.002 | 0.00 | 5 |
| | SD | 0.08 | 0.15 | 0.07 | 0.05 | 0.01 | 0.004 | 0.004 | 0.00 | |
| | mean no. of worms per rat | | | | | 41 | | | | |
| challenge controls Expt 3 Gp.C3 | Mean | 0.31 | 0.43 | 0.22 | 0.03 | 0.008 | 0.00 | 0.00 | 0.003 | 6 |
| | SD | 0.08 | 0.18 | 0.12 | 0.05 | 0.02 | 0.00 | 0.00 | 0.008 | |
| | mean no. of worms per rat | | | | | 60 | | | | |

Table 4:2(iii)

Distribution of worms along the intestine in Expt 4

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.41 | 0.23 | 0.22 | 0.06 | 0.04 | 0.03 | 0.00 | 0.00 | 10 |
| | SD | 0.36 | 0.21 | 0.30 | 0.09 | 0.07 | 0.05 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 12 | | | | | | | | |
| heterologous challenge Gp.D | Mean | 0.15 | 0.44 | 0.14 | 0.18 | 0.02 | 0.03 | 0.02 | 0.01 | 7 |
| | SD | 0.13 | 0.36 | 0.14 | 0.37 | 0.03 | 0.07 | 0.03 | 0.02 | |
| | mean no. of worms per rat | 6 | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.41 | 0.36 | 0.13 | 0.07 | 0.03 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.16 | 0.03 | 0.08 | 0.04 | 0.03 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 49 | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C2 | Mean | 0.29 | 0.35 | 0.21 | 0.12 | 0.02 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.13 | 0.12 | 0.08 | 0.11 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 36 | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C3 | Mean | 0.24 | 0.38 | 0.24 | 0.11 | 0.03 | 0.01 | 0.00 | 0.00 | 6 |
| | SD | 0.06 | 0.07 | 0.04 | 0.04 | 0.03 | 0.03 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 44 | | | | | | | | |

Table 4:2(iv)

Distribution of worms along the intestine in Expt 5

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.50 | 0.06 | 0.10 | 0.15 | 0.04 | 0.07 | 0.02 | 0.05 | 9 |
| | SD | 0.33 | 0.09 | 0.13 | 0.17 | 0.06 | 0.17 | 0.05 | 0.10 | |
| | mean no. of worms per rat | 8 | | | | | | | | |
| heterologous challenge Gp.D | Mean | 0.32 | 0.05 | 0.05 | 0.18 | 0.00 | 0.06 | 0.04 | 0.18 | 8 |
| | SD | 0.33 | 0.10 | 0.10 | 0.36 | 0.00 | 0.08 | 0.06 | 0.35 | |
| | mean no. of worms per rat | 3 | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.35 | 0.39 | 0.14 | 0.11 | 0.02 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.06 | 0.04 | 0.07 | 0.08 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 52 | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C2 | Mean | 0.37 | 0.27 | 0.21 | 0.13 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.11 | 0.10 | 0.05 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 54 | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C3 | Mean | 0.44 | 0.30 | 0.16 | 0.10 | 0.02 | 0.004 | 0.00 | 0.00 | 5 |
| | SD | 0.11 | 0.10 | 0.02 | 0.04 | 0.03 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 41 | | | | | | | | |

Table 4:2(v)

Distribution of worms along the intestine in Expt 6

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.21 | 0.31 | 0.25 | 0.15 | 0.01 | 0.03 | 0.03 | 0.00 | 6 |
| | SD | 0.27 | 0.40 | 0.18 | 0.21 | 0.03 | 0.09 | 0.09 | 0.00 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| heterologous challenge Gp.D | Mean | 0.57 | 0.27 | 0.12 | 0.03 | 0.003 | 0.00 | 0.00 | 0.00 | 10 |
| | SD | 0.09 | 0.10 | 0.08 | 0.03 | 0.010 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 30 | | | | | |
| priming controls Gp.C1 | Mean | 0.57 | 0.33 | 0.10 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.06 | 0.05 | 0.10 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 42 | | | | | |
| challenge heterogonic S.ratti controls Gp.C2 | Mean | 0.51 | 0.32 | 0.11 | 0.05 | 0.01 | 0.004 | 0.00 | 0.00 | 5 |
| | SD | 0.09 | 0.04 | 0.05 | 0.03 | 0.02 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 52 | | | | | |
| challenge homogonic S.ratti controls Gp.C3 | Mean | 0.38 | 0.33 | 0.20 | 0.08 | 0.00 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.07 | 0.05 | 0.07 | 0.07 | 0.00 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 65 | | | | | |

Table 4:2(vi)

Distribution of worms along the intestine in Expt 7

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.62 | 0.10 | 0.09 | 0.12 | 0.00 | 0.00 | 0.00 | 0.07 | 7 |
| | SD | 0.49 | 0.25 | 0.16 | 0.21 | 0.00 | 0.00 | 0.00 | 0.19 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| heterologous challenge Gp.D | Mean | 0.64 | 0.18 | 0.11 | 0.04 | 0.02 | 0.00 | 0.04 | 0.004 | 10 |
| | SD | 0.23 | 0.16 | 0.09 | 0.05 | 0.07 | 0.00 | 0.02 | 0.01 | |
| | mean no. of worms per rat | | | | 15 | | | | | |
| priming controls Gp.C1 | Mean | 0.42 | 0.39 | 0.12 | 0.06 | 0.02 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.08 | 0.16 | 0.06 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 41 | | | | | |
| challenge heterogonic S.ratti controls Gp.C2 | Mean | 0.33 | 0.46 | 0.14 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.02 | 0.10 | 0.10 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 36 | | | | | |
| challenge homogonic S.ratti controls Gp.C3 | Mean | 0.38 | 0.31 | 0.14 | 0.12 | 0.06 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.07 | 0.10 | 0.05 | 0.06 | 0.07 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 51 | | | | | |

Table 4:2(vii)

Distribution of worms along the intestine in Expt 8

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous | Mean | 0.86 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 8 |
| challenge | SD | 0.14 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.B | mean no. of worms per rat | | | | | 6 | | | | |
| heterologous | Mean | 0.36 | 0.35 | 0.19 | 0.05 | 0.01 | 0.002 | 0.00 | 0.00 | 12 |
| challenge | SD | 0.11 | 0.11 | 0.08 | 0.05 | 0.02 | 0.010 | 0.00 | 0.00 | |
| Gp.D | mean no. of worms per rat | | | | | 44 | | | | |
| residual | Mean | 0.91 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 8 |
| S.venezuelensis | SD | 0.13 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.E | mean no. of worms per rat | | | | | 6 | | | | |
| priming | Mean | 0.87 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| controls | SD | 0.19 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.C1 | mean no. of worms per rat | | | | | 5 | | | | |
| challenge | Mean | 0.87 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| S.venezuelensis | SD | 0.25 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| controls, Gp.C2 | mean no. of worms per rat | | | | | 2 | | | | |
| challenge | Mean | 0.55 | 0.30 | 0.09 | 0.06 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| homogonic | SD | 0.23 | 0.21 | 0.06 | 0.08 | 0.02 | 0.00 | 0.00 | 0.00 | |
| S.ratti | mean no. of worms per rat | | | | | 22 | | | | |
| controls, Gp.C3 | | | | | | | | | | |

Table 4:2(viii)

Distribution of worms along the intestine in Expt 9

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous | Mean | 0.89 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10 |
| challenge | SD | 0.10 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.B | mean no. of worms per rat | | | | | 10 | | | | |
| heterologous | Mean | 0.34 | 0.40 | 0.18 | 0.06 | 0.01 | 0.01 | 0.00 | 0.00 | 10 |
| challenge | SD | 0.07 | 0.09 | 0.09 | 0.03 | 0.01 | 0.02 | 0.00 | 0.00 | |
| Gp.D | mean no. of worms per rat | | | | | 64 | | | | |
| residual | Mean | 0.79 | 0.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 9 |
| S.venezuelensis | SD | 0.31 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.E | mean no. of worms per rat | | | | | 5 | | | | |
| priming | Mean | 0.72 | 0.28 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| controls | SD | 0.44 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.C1 | mean no. of worms per rat | | | | | 2 | | | | |
| challenge | Mean | 0.97 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| S.venezuelensis | SD | 0.08 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| controls, Gp.C2 | mean no. of worms per rat | | | | | 4 | | | | |
| challenge | Mean | 0.33 | 0.38 | 0.16 | 0.08 | 0.02 | 0.004 | 0.00 | 0.00 | 5 |
| heterogonic | SD | 0.09 | 0.17 | 0.05 | 0.07 | 0.02 | 0.010 | 0.00 | 0.00 | |
| S.ratti | mean no. of worms per rat | | | | | 33 | | | | |
| controls, Gp.C3 | | | | | | | | | | |

## 4:3 Third-stage larval output post-challenge for Expt 5

Table 4:3(i)

| Treatment | Day | L3 output/gm. faeces | | | Theoretical number Of L3 which could be produced by all faeces collected | | |
|---|---|---|---|---|---|---|---|
| | | Mean L3/gm. faeces | n | SD | Total no. L3 produced | n | SD |
| homogonic | 6 | 193.6 | 3 | 92.6 | 2056.2 | 3 | 1350.9 |
| S.ratti | 7 | 133.6 | 3 | 104.8 | 1200.6 | 3 | 1006.9 |
| challenge | 8 | 197.8 | 3 | 203.6 | 314.6 | 3 | 398.8 |
| controls | | | | | | | |
| heterogonic | 6 | 82.3 | 3 | 46.5 | 895.2 | 3 | 465.3 |
| S.ratti | 7 | 209.3 | 3 | 180.5 | 2410.8 | 3 | 2159.3 |
| challenge | 8 | 65.4 | 3 | 51.8 | 150.5 | 3 | 34.2 |
| controls | | | | | | | |
| homologous | 6 | 0.7 | 3 | 1.0 | 11.0 | 3 | 17.0 |
| challenge | 7 | 31.4 | 3 | 39.8 | 320.3 | 3 | 510.5 |
| | 8 | 21.0 | 3 | 17.9 | 90.0 | 3 | 85.4 |
| heterologous | 6 | 0.0 | 3 | 0.0 | | | |
| challenge | 7 | 0.0 | 3 | 0.0 | | | |
| | 8 | 0.2 | 3 | 0.3 | 0.7 | 3 | 1.2 |

## 4:4 Resistance quotients for Expts 4-9

Table 4:4(i)

Resistance quotients

| Expt | Treatment | Worm data | | | Egg data | | |
|---|---|---|---|---|---|---|---|
| | | $ReQ_w$ | n | SE | $ReQ_e$ | n | SE |
| 4 | homologous challenge | 0.70 | 10 | 0.09 | 0.81 | 80 | 0.03 |
| | heterologous challenge | 0.88 | 10 | 0.05 | 0.73 | 50 | 0.04 |
| 5 | homologous challenge | 0.84 | 10 | 0.04 | 0.83 | 61 | 0.02 |
| | heterologous challenge | 0.93 | 9 | 0.03 | 0.76 | 22 | 0.07 |
| 6 | homologous challenge | 0.93 | 9 | 0.03 | 0.81 | 20 | 0.04 |
| | heterologous challenge | 0.52 | 10 | 0.03 | 0.66 | 154 | 0.02 |
| 7 | homologous challenge | 0.97 | 10 | 0.01 | 0.89 | 10 | 0.04 |
| | heterologous challenge | 0.70 | 10 | 0.05 | 0.63 | 100 | 0.02 |
| 8 | homologous challenge | -1.59 | 9 | 0.61 | | | |
| | heterologous challenge | -0.85 | 10 | 0.18 | | | |
| 9 | homologous challenge | -1.39 | 9 | 0.44 | | | |
| | heterologous challenge | -0.70 | 10 | 0.12 | | | |

Table 5:1(i)

Mode Of Infection, Expt 1

| Treatment | mean proportion of the dose recovered | n | SD | age of rats | mean exact dose |
|---|---|---|---|---|---|
| subcutaneous injection | 0.091 | 4 | 0.107 | Adult | 94.75 |
| skin-application | 0.092 | 4 | 0.119 | Adult | 97.75 |

Table 5:1(ii)

Mode of anaesthertizing rats, Expt 2

| Treatment | mean proportion of the dose recovered | n | SD | age of rats | mean exact dose |
|---|---|---|---|---|---|
| Sagatal | 0.034 | 4 | 0.023 | Adult | 97.00 |
| ether | 0.090 | 4 | 0.072 | Adult | 94.75 |

Table 5:1(iii)

Increasing infection dose, Expt 3

| Treatment | mean proportion of the dose recovered | n | SD | age of rats | mean no. of eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|---|
| infection with 2000 infective larvae | 0.290 | 6 | 0.058 | adult | 7.037 | 108 | 0.91 |

Table 5:1(iv)

Age of host, Expt 4

| Treatment | mean proportion of the dose recovered | n | SD | mean exact dose |
|---|---|---|---|---|
| 3 week old rats | 0.298 | 4 | 0.080 | 96.00 |
| adult rats | 0.013 | 4 | 0.019 | |

Table 5:1(v)

Age of host: using labelled larvae, 72 hours post-infection, Expt 5

| Treatment | mean prop. of the dose recovered | n | SD | mean prop. of the dose in the anterior Intestine | SD | mean prop. of the dose in the posterior Intestine | SD |
|---|---|---|---|---|---|---|---|
| homogonic S.ratti adult rats | 0.321 | 4 | 0.059 | 0.274 | 0.041 | 0.047 | 0.020 |
| homogonic S.ratti 3 week old rats | 0.326 | 3 | 0.062 | 0.275 | 0.036 | 0.054 | 0.029 |
| S.venezuelensis adult rats | 0.003 | 5 | 0.004 | 0.0008 | 0.002 | 0.003 | 0.003 |
| S.venezuelensis 3 week old rats | 0.180 | 4 | 0.048 | 0.175 | 0.044 | 0.004 | 0.004 |

| | | | | |
|---|---|---|---|---|
| S.ratti | 2500L3 | 5000cpm | thus 2.0cpm/L3 | Background 238cpm |
| S.venezuelensis | 2500L3 | 4745cpm | thus 2.9cpm/L3 | Background 219cpm |

Table 5:1(vi)

Age of host: using labelled larvae, days 3 and 8 post-infection, Expt 6

| Treatment | mean prop. of the dose recovered | n | SD | mean prop. of the dose in the anterior intestine | SD | mean prop. of the dose in the posterior intestine | SD |
|---|---|---|---|---|---|---|---|
| S.venezuelensis adult rats 72 hours | 0.016 | 7 | 0.007 | 0.012 | 0.005 | 0.004 | 0.002 |
| S.venezuelensis adult rats day 8 | 0.031 | 8 | 0.007 | 0.029 | 0.007 | 0.002 | 0.001 |
| S.venezuelensis adult rats day 8 counted worms | 0.103 | 8 | 0.028 | 0.103 | 0.03 | 0.000 | 0.000 |

S.venezuelensis 2030L3 7511cpm thus 3.7cpm/L3 Background 227cpm

Table 5:2(i)

Distribution of worms along the intestine in Expt 1

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| subcutaneous injection | Mean | 0.82 | 0.13 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.29 | 0.19 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 9 | | | | | |
| skin-application | Mean | 0.93 | 0.04 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.06 | 0.08 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 9 | | | | | |

Table 5:2(ii)

Distribution of worms along the intestine in Expt 2

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| Sagatal anaesthesia | Mean | 0.76 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.16 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| ether anaesthesia | Mean | 0.92 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.12 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 9 | | | | | |

Table 5:2(iii)

Distribution of worms along the intestine in Expt 3

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| dose Of 2000L3 | Mean | 0.59 | 0.30 | 0.10 | 0.01 | 0.003 | 0.002 | 0.00 | 0.00 | 6 |
| | SD | 0.06 | 0.03 | 0.03 | 0.01 | 0.004 | 0.004 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 580 | | | | | |

Table 5:1(iv)

Distribution of worms along the intestine in Expt 4

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 week old rats | Mean | 0.69 | 0.28 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.10 | 0.06 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 29 | | | | | |
| adult rats | Mean | 0.75 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2 |
| | SD | 0.18 | 0.00 | 0.35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |

## 6:1 Summary of egg and worm data

Table 6:1(i)

Priming rats with heat-killed third-stage homogonic S.ratti larvae, Expt 1

| Treatment | Mean proportion of the dose recovered | n | SD |
|---|---|---|---|
| homologous challenge | 0.179 | 15 | 0.084 |
| day 20 skin-application challenge controls | 0.211 | 15 | 0.112 |
| day 8 challenge injection controls | 0.436 | 5 | 0.089 |
| mean exact dose | skin-application | 95.47 | |
| | injection | 80.00 | |

Table 6:1(ii)

Day 4 post-infection with transferred S.venezuelensis worms, Expt 2

| Treatment | mean proportion of the dose recovered | n | SD | mean exact dose |
|---|---|---|---|---|
| day 4 | 0.321 | 4 | 0.069 | 90 |
| mortality 0% | | | | |

Table 6:1(iv)

Homologous vs heterologous challenge: homogonic S.ratti worms priming rats, Expt 8

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/ worm/rat | n | SD | mean no. of eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| homologous challenge | 0.093 | 7 | 0.063 | 1.740 | 6 | 0.838 | 2.526 | 19 | 4.384 |
| heterologous challenge | 0.092 | 7 | 0.039 | 2.417 | 6 | 1.191 | 2.385 | 26 | 2.041 |
| priming homogonic S.ratti controls | 0.333 | 5 | 0.333 | 7.261 | 4 | 0.603 | 7.419 | 31 | 1.057 |
| day 8 challenge homogonic S.ratti controls | 0.251 | 5 | 0.251 | 7.621 | 5 | 1.005 | 7.000 | 31 | 1.648 |
| day 8 challenge heterogonic S.ratti controls | 0.195 | 3 | 0.101 | 5.708 | 3 | 1.450 | 4.833 | 12 | 1.946 |

mean exact dose — priming: homogonic S.ratti 44.32; challenge: homogonic S.ratti 47.00, heterogonic S.ratti 46.50

mortality 0%

Table 6:1(iii)

Homologous challenge: homogonic S.ratti worms priming rats, Expt 7

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/ worm/rat | n | SD | mean no. of eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| homologous challenge day 4 | 0.229 | 5 | 0.102 | 2.947 | 5 | 1.699 | 3.656 | 32 | 1.945 |
| homologous challenge day 8 | 0.065 | 5 | 0.052 | 1.367 | 4 | 0.427 | 1.444 | 9 | 0.882 |
| day 4 priming control | 0.359 | 4 | 0.173 | 7.521 | 4 | 0.625 | 7.500 | 26 | 1.068 |
| day 4 challenge control | 0.237 | 5 | 0.198 | 6.387 | 4 | 0.592 | 6.607 | 28 | 1.367 |
| day 8 challenge control | 0.397 | 5 | 0.224 | 6.870 | 5 | 0.912 | 7.267 | 30 | 1.337 |

mean exact dose — priming 47.4; challenge 47.8

mortality 0%

Table 6:1(v)

Priming with a trickle infection of S.venezuelensis: homologous vs heterologous challenge, Expt 9

Table 6:1(viii)a

Adult recoveries, Expt 9

| Treatment | S.venezuelensis | | | S.ratti | | |
|---|---|---|---|---|---|---|
| | Mean proportion of the dose recovered | n | SD | Mean proportion of the dose recovered | n | SD |
| 1 week of trickle infection | 0.068 | 5 | 0.068 | | | |
| 3 weeks of trickle infection | 0.017 | 5 | 0.015 | | | |
| 5 weeks of trickle infection | 0.022 | 5 | 0.017 | | | |
| 6 weeks of trickle infection | 0.022 | 5 | 0.018 | | | |
| heterologous challenge | 0.019 | 6 | 0.009 | 0.416 | 5 | 0.105 |
| challenge homogonic S.ratti controls | | | | 0.448 | 5 | 0.061 |
| challenge homogonic S.ratti sham controls | | | | 0.466 | 5 | 0.183 |

| | | | |
|---|---|---|---|
| mean exact dose | S.venezuelensis | 1 week of trickle | 23.2 |
| | | 3 weeks of trickle | 72.2 |
| | | 5 weeks of trickle | 117.6 |
| | | 6 weeks of trickle | 147.7 |
| | S.ratti | skin-application | 97.7 |

Table 6:1(v)b

Egg data, S.venezuelensis, Expt 9

| Treatment | mean no. eggs in utero/ worm/rat | n | SD | mean no. eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|
| 1 week of trickle infection | 5.500 | 2 | 0.709 | 5.500 | 2 | 0.707 |
| 3 weeks of trickle infection | 6.778 | 3 | 1.072 | 6.600 | 5 | 1.342 |
| 5 weeks of trickle infection | 4.100 | 4 | 1.070 | 3.667 | 6 | 1.633 |
| 6 weeks of trickle infection | 4.400 | 5 | 2.278 | 4.300 | 10 | 1.767 |
| heterologous challenge | 5.083 | 4 | 0.484 | 5.111 | 9 | 1.274 |

Table 6:1(v)c

Egg data, homogonic S.ratti, Expt 9

| Treatment | mean no. eggs in utero/ worm/rat | n | SD | mean no. eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|
| heterologous challenge | 6.024 | 5 | 0.613 | 6.000 | 51 | 1.223 |
| challenge controls | 6.028 | 5 | 0.506 | 6.109 | 55 | 1.208 |
| challenge sham controls | 6.140 | 5 | 0.447 | 6.111 | 45 | 1.071 |

Table 6:1(vi)

Priming with a trickle infection of S.venezuelensis: homologous vs heterologous challenge, Expt 10

Table 6:1(vi)a

Adult recoveries, Expt 10

| Treatment | S.venezuelensis | | | S.ratti | | |
|---|---|---|---|---|---|---|
| | mean proportion of the dose recovered | n | SD | mean proportion of the dose recovered | n | SD |
| 5 weeks of trickle infection | 0.010 | 5 | 0.007 | | | |
| 7 weeks of trickle infection | 0.001 | 5 | 0.003 | | | |
| 9 weeks of trickle infection | 0.000 | 5 | 0.000 | | | |
| 10 weeks of trickle infection | 0.003 | 5 | 0.003 | | | |
| heterologous challenge | 0.002 | 6 | 0.003 | 0.422 | 6 | 0.418 |
| challenge homogonic S.ratti sham controls | | | | 0.442 | 5 | 0.134 |
| challenge homogonic S.ratti controls | | | | 0.513 | 5 | 0.093 |

| | | | |
|---|---|---|---|
| mean exact dose | S.venezuelensis | 5 weeks of trickle | 119.0 |
| | | 7 weeks of trickle | 169.6 |
| | | 9 weeks of trickle | 219.6 |
| | | 10 weeks of trickle | 243.9 |
| | S.ratti | skin-application | 95.8 |

Table 6:1(vi)b

Egg data, S.venezuelensis, Expt 10

| Treatment | mean no. eggs in utero/ worm/rat | n | SD | mean no. eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|
| 5 weeks of trickle infection | 6.700 | 1 | - | 6.700 | 1 | - |
| 7 weeks of trickle infection | - | - | - | - | - | - |
| 9 weeks of trickle infection | - | - | - | - | - | - |
| heterologous challenge | 5.000 | 1 | - | 5.000 | 1 | - |

Table 6:1(vi)c

Egg data, S.ratti, Expt 10

| Treatment | mean no. eggs in utero/ worm/rat | n | SD | mean no.eggs in utero/ worm | n | SD |
|---|---|---|---|---|---|---|
| heterologous challenge | 5.950 | 6 | 0.740 | 6.056 | 36 | 1.604 |
| challenge controls | 6.323 | 5 | 0.303 | 6.393 | 28 | 1.068 |
| challenge sham controls | 7.085 | 5 | 0.552 | 7.000 | 36 | 1.005 |

6:2 Distribution of worms along the intestine in Expts 2-11

Table 6:2(i)

Expt 2

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 4 | Mean | 0.50 | 0.39 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| S.venezuelensis | SD | 0.43 | 0.39 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 29 | | | | | | | | |

Table 6:2(ii)

Expt 3, S.venezuelensis

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 4 | Mean | 0.69 | 0.24 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.31 | 0.20 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 11 | | | | | | | | |
| day 14 | Mean | 0.72 | 0.08 | 0.15 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.41 | 0.11 | 0.34 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 6 | | | | | | | | |
| day 21 | Mean | 0.91 | 0.07 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.22 | 0.11 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 7 | | | | | | | | |

Table 6:2(iii)

Expt 4, heterogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 4 | Mean | 0.33 | 0.35 | 0.14 | 0.17 | 0.008 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.34 | 0.35 | 0.11 | 0.41 | 0.002 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 14 | | | | | | | | |
| day 14 | Mean | 0.47 | 0.19 | 0.16 | 0.09 | 0.07 | 0.03 | 0.00 | 0.00 | 6 |
| | SD | 0.28 | 0.18 | 0.15 | 0.17 | 0.12 | 0.04 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 9 | | | | | | | | |
| day 21 | Mean | 0.24 | 0.35 | 0.29 | 0.04 | 0.09 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.27 | 0.22 | 0.46 | 0.10 | 0.14 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 5 | | | | | | | | |

Table 6:2(iv)

Expt 5, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 4 | Mean | 0.45 | 0.31 | 0.18 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.36 | 0.19 | 0.25 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 14 | | | | | | | | |
| day 14 | Mean | 0.69 | 0.25 | 0.06 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.32 | 0.25 | 0.14 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 15 | | | | | | | | |
| day 21 | Mean | 0.76 | 0.20 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.29 | 0.23 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 6 | | | | | | | | |

Table 6:2(v)

Expt 6, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 4 | Mean | 0.81 | 0.12 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.25 | 0.24 | 0.05 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 20 | | | | | | | | |
| day 14 | Mean | 0.82 | 0.12 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.27 | 0.19 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 13 | | | | | | | | |
| day 21 | Mean | 0.96 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.10 | 0.05 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 5 | | | | | | | | |

Table 6:2(vi)

Expt 7, Homologous challenge with homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge day 4 | Mean | 0.69 | 0.16 | 0.07 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.18 | 0.16 | 0.07 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 11 | | | | | |
| homologous challenge day 8 | Mean | 0.44 | 0.15 | 0.14 | 0.05 | 0.13 | 0.05 | 0.00 | 0.05 | 4 |
| | SD | 0.52 | 0.17 | 0.19 | 0.10 | 0.25 | 0.10 | 0.00 | 0.10 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| priming controls Gp.C1 | Mean | 0.78 | 0.09 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.44 | 0.14 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 17 | | | | | |
| challenge controls day 4, Gp.C2 | Mean | 0.90 | 0.07 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.22 | 0.16 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 11 | | | | | |
| challenge controls day 8, Gp.C2 | Mean | 0.78 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.22 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 19 | | | | | |

Table 6:2(vii)

Expt 8

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.68 | 0.20 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.32 | 0.24 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 4 | | | | | |
| heterologous challenge Gp.D | Mean | 0.51 | 0.09 | 0.24 | 0.09 | 0.03 | 0.00 | 0.04 | 0.00 | 7 |
| | SD | 0.38 | 0.16 | 0.38 | 0.12 | 0.08 | 0.00 | 0.09 | 0.00 | |
| | mean no. of worms per rat | | | | 4 | | | | | |
| priming homomgonic S.ratti controls Gp.C1 | Mean | 0.85 | 0.10 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.31 | 0.19 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 15 | | | | | |
| challenge homogonic S.ratti controls Gp.C2 | Mean | 0.73 | 0.23 | 0.07 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.32 | 0.23 | 0.11 | 0.05 | 0.05 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 12 | | | | | |
| challenge heterogonic S.ratti controls Gp.C3 | Mean | 0.63 | 0.37 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.44 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 9 | | | | | |

Table 6:2(viii)

Expt 9

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 week of trickle infection | Mean | 0.75 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.25 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 2 | | | | | |
| 3 weeks of trickle infection | Mean | 0.92 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.17 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| 5 weeks of trickle infection | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| 6 weeks of trickle infection | Mean | 0.96 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.10 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| heterologous challenge residual S.venezuelensis | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| heterologous challenge homogonic S.ratti | Mean | 0.49 | 0.35 | 0.12 | 0.03 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.07 | 0.04 | 0.05 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 41 | | | | | |
| challenge homogonic S.ratti controls | Mean | 0.53 | 0.36 | 0.08 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.03 | 0.11 | 0.08 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 44 | | | | | |
| challenge homogonic S.ratti sham controls | Mean | 0.64 | 0.28 | 0.08 | 0.007 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.10 | 0.07 | 0.04 | 0.100 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 46 | | | | | |

Table 6:2(ix)

Expt 10

| Treatment | | Proportion of the worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 weeks of trickle infection | Mean | 0.88 | 0.00 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.25 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| 7 weeks of trickle infection | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| 10 weeks of trickle infection | Mean | 0.83 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.29 | 0.00 | 0.29 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| heterologous challenge residual S.veneuelensis | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| heterologous challenge homogonic S.ratti | Mean | 0.45 | 0.39 | 0.12 | 0.02 | 0.01 | 0.005 | 0.00 | 0.00 | 6 |
| | SD | 0.08 | 0.08 | 0.04 | 0.02 | 0.02 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 40 | | | | | |
| challenge homogonic S.ratti controls | Mean | 0.42 | 0.36 | 0.16 | 0.04 | 0.02 | 0.004 | 0.00 | 0.00 | 5 |
| | SD | 0.19 | 0.08 | 0.08 | 0.03 | 0.02 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 40 | | | | | |
| challenge homogonic S.ratti sham controls | Mean | 0.49 | 0.38 | 0.11 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.04 | 0.09 | 0.07 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 49 | | | | | |

Table 6:2(x)

Expt 11

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.75 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.37 | 0.37 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| heterologous challenge homogonic *S.ratti*, Gp.D | Mean | 0.20 | 0.39 | 0.29 | 0.11 | 0.02 | 0.00 | 0.00 | 0.00 | 8 |
| | SD | 0.09 | 0.11 | 0.15 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 34 | | | | | |
| *S.venezuelensis* present in heterologous homogonic *S.ratti* treatment | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 3 | | | | | |
| heterologous challenge heterogonic *S.ratti*, Gp.E | Mean | 0.24 | 0.23 | 0.28 | 0.22 | 0.04 | 0.01 | 0.00 | 0.00 | 7 |
| | SD | 0.14 | 0.08 | 0.13 | 0.14 | 0.06 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 33 | | | | | |
| *S.venezuelensis* present in heterologous heterogonic *S.ratti* treatment | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 1 | | | | | |
| priming controls Gp.C1 | Mean | 0.72 | 0.26 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.21 | 0.19 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 83 | | | | | |
| challenge controls *S.venezuelensis* Gp.C2 | Mean | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| challenge homogonic *S.ratti* controls, Gp.C3 | Mean | 0.33 | 0.34 | 0.22 | 0.12 | 0.01 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.18 | 0.09 | 0.15 | 0.08 | 0.01 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 47 | | | | | |
| challenge heterogonic *S.ratti* controls, Gp.C4 | Mean | 0.21 | 0.47 | 0.16 | 0.12 | 0.04 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.07 | 0.05 | 0.04 | 0.05 | 0.04 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | | | | 44 | | | | | |

## 6:3 Resistance ratios for worm and egg data

Table 6:3(i)

Expt 8, Egg data

| Treatment | Group | ReQe | n | SD |
|---|---|---|---|---|
| homologous challenge | A | 0.771 | 6 | 0.045 |
| heterologous challenge | B | 0.506 | 6 | 0.2092 |

Table 6:3(ii)

Expt 11, Worm data

| Treatment | Group | ReQw | n | SD |
|---|---|---|---|---|
| homologous challenge | A | 0.366 | 7 | 0.805 |
| heterologous challenge homogonic *S.ratti* | B | 0.265 | 8 | 0.157 |
| heterologous challenge heterogonic *S.ratti* | D | 0.652 | 7 | 0.093 |

Table 6:3(iii)

Expt 11, Egg data

| Treatment | Group | ReQe | n | SD |
|---|---|---|---|---|
| homologous challenge | A | 0.718 | 4 | 0.242 |
| heterologous challenge homogonic *S.ratti* | B | 0.363 | 7 | 0.1202 |
| heterologous challenge heterogonic *S.ratti* | D | 0.665 | 7 | 0.1403 |

7:1 Summary of worm data

Table 7:1(i)

Homologous challenge, homogonic strain of S.ratti: kill day 8, Expt 1

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| secondary challenge | 0.163 | 6 | 0.09 | 1.188 | 69 | 1.82 | 3.100 | 5 | 0.93 |
| immune mesenteric lymph node cells | 0.443 | 5 | 0.10 | 6.543 | 92 | 1.14 | 6.528 | 5 | 0.31 |
| normal mesenteric lymph node cells | 0.525 | 5 | 0.13 | 7.134 | 97 | 1.20 | 7.144 | 5 | 0.34 |
| day 25 post-primary infection | 0.182 | 6 | 0.02 | 5.053 | 95 | 1.95 | 5.152 | 6 | 0.83 |
| priming controls | 0.599 | 4 | 0.10 | 7.688 | 80 | 1.01 | 7.688 | 4 | 0.25 |
| challenge controls | 0.550 | 5 | 0.10 | 7.113 | 80 | 1.68 | 7.210 | 5 | 0.39 |
| mean exact dose | priming | | | 97.81 | | | | | |
| | challenge | | | 97.48 | | | | | |

Table 7:1(ii)

Homologous challenge, homogonic strain of S.ratti; kill day 8, Expt 2

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| secondary infection | 0.141 | 5 | 0.06 | 1.958 | 48 | 1.14 | 1.749 | 5 | 0.89 |
| immune mesenteric lymph node cells | 0.681 | 5 | 0.09 | 6.053 | 75 | 1.20 | 5.910 | 5 | 0.36 |
| normal mesenteric lymph node cells | 0.754 | 4 | 0.06 | 6.708 | 96 | 1.35 | 6.906 | 4 | 0.68 |
| day 25 post-primary infection | 0.174 | 5 | 0.06 | 4.045 | 66 | 1.69 | 4.190 | 5 | 0.70 |
| primary controls | 0.582 | 3 | 0.03 | 7.719 | 57 | 0.96 | 7.725 | 3 | 0.24 |
| challenge controls | 0.653 | 7 | 0.18 | 6.975 | 119 | 1.27 | 7.058 | 7 | 0.54 |
| mean exact dose | priming | | | 97.46 | | | | | |
| | challenge | | | 88.86 | | | | | |

Table 7:1(iv)

Homologous challenge, homogonic strain of S.ratti, kill day 16, Expt 4

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of eggs in utero/worm/ rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| immune mesenteric lymph node cells 2000L3 donors | 0.027 | 3 | 0.02 | 1.571 | 7 | 1.40 | 1.750 | 3 | 1.25 |
| immune mesenteric lymph node cells <100L3 donors | 0.137 | 5 | 0.06 | 3.918 | 49 | 1.86 | 3.929 | 5 | 0.54 |
| priming controls | 0.655 | 3 | 0.09 | 7.283 | 60 | 1.29 | 7.283 | 3 | 0.03 |
| challenge day 8 controls | 0.567 | 4 | 0.08 | 7.038 | 59 | 1.32 | 7.030 | 4 | 0.20 |
| challenge day 16 controls | 0.292 | 5 | 0.07 | 6.494 | 85 | 1.65 | 6.441 | 5 | 0.52 |

| | | |
|---|---|---|
| mean exact dose | priming | 95.20 |
| | challenge | 97.12 |

Table 7:1(iii)

Increasing infection dose in donors of immune cells: homologous challenge homogonic S.ratti, kill day 8, Expt 3

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of eggs in utero/worm/ rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| immune mesenteric lymph node cells 4000L3 donors | 0.463 | 7 | 0.05 | 6.266 | 128 | 1.38 | 6.271 | 7 | 0.53 |
| immune mesenteric lymph node cells 1000L3 donors | 0.429 | 6 | 0.11 | 5.566 | 113 | 1.40 | 5.553 | 6 | 0.41 |
| day 25 1000L3 post-primary infection | 0.0003 | 6 | 0.0008 | 0.000 | 1 | 0.00 | 0.000 | 1 | 0.00 |
| day 25 4000L3 post-primary infection | 0.00005 | 6 | 0.0001 | 0.000 | 2 | 0.00 | 0.000 | 1 | 0.00 |
| priming controls | 0.648 | 5 | 0.06 | 7.380 | 100 | 1.18 | 7.380 | 5 | 0.14 |
| challenge controls | 0.516 | 5 | 0.12 | 6.972 | 72 | 1.13 | 6.970 | 5 | 0.37 |

| | | |
|---|---|---|
| mean exact dose | priming | 93.00 |
| | challenge | 94.47 |

Table 7:1(v)

Homologous challenge, homogonic strain of S.ratti, kill day 16, Expt 5

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| immune mesenteric lymph node cells 2000L3 donors | 0.121 | 6 | 0.07 | 3.146 | 48 | 2.32 | 2.810 | 6 | 1.34 |
| immune mesenteric lymph node cells <100L3 donors | 0.304 | 5 | 0.06 | 5.862 | 87 | 1.37 | 5.825 | 5 | 0.48 |
| priming controls | 0.562 | 3 | 0.15 | 7.980 | 50 | 1.15 | 7.933 | 4 | 0.18 |
| challenge day 8 controls | 0.584 | 4 | 0.10 | 1.231 | 65 | 1.04 | 7.220 | 4 | 0.18 |
| challenge day 16 controls | 0.441 | 4 | 0.08 | 1.288 | 80 | 1.25 | 7.288 | 4 | 0.21 |

mean exact dose — priming 93.80; challenge 96.79

Table 7:1(vi)

Homologous challenge, heterogonic strain of S.ratti, kill day 16, Expt 6

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of eggs in utero/worm/rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| immune mesenteric lymph node cells 2000L3 donors | 0.132 | 6 | 0.05 | 2.375 | 56 | 1.40 | 2.422 | 6 | 0.71 |
| immune mesenteric lymph node cells <100L3 donors | 0.172 | 4 | 0.07 | 2.709 | 55 | 2.00 | 2.561 | 4 | 1.06 |
| priming controls | 0.483 | 3 | 0.06 | 6.286 | 49 | 1.21 | 6.281 | 3 | 0.27 |
| challenge day 8 controls | 0.517 | 4 | 0.05 | 6.333 | 45 | 1.40 | 6.304 | 4 | 0.47 |
| challenge day 16 controls | 0.340 | 5 | 0.09 | 5.212 | 113 | 1.40 | 4.990 | 5 | 0.59 |

mean exact dose — priming 97.40; challenge 98.42

Table 7:1(vii)

Homologous challenge, heterogonic strain of S.ratti, kill day 16, Expt 7

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm | n | SD | mean no. of Eggs in utero/worm/ rat | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| immune mesenteric lymph node cells 2000L3 donors | 0.090 | 7 | 0.05 | 3.033 | 30 | 1.85 | 3.367 | 7 | 1.42 |
| immune mesenteric lymph node cells <100L3 donors | 0.107 | 4 | 0.09 | 3.630 | 27 | 1.50 | 4.500 | 4 | 1.24 |
| priming controls | 0.446 | 4 | 0.12 | 6.093 | 43 | 1.25 | 6.050 | 4 | 0.68 |
| challenge controls | 0.277 | 4 | 0.10 | 7.235 | 34 | 1.08 | 7.075 | 4 | 0.40 |

| | | |
|---|---|---|
| mean exact dose | priming | 97.64 |
| | challenge | 93.88 |

7:2 Distribution of adults along the intestine in Expts 1-13

Table 7:2(i)

Expt 1, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| secondary | Mean | 0.35 | 0.11 | 0.08 | 0.11 | 0.17 | 0.12 | 0.03 | 0.03 | 6 |
| infection | SD | 0.38 | 0.09 | 0.08 | 0.13 | 0.11 | 0.10 | 0.06 | 0.06 | |
| Gp.B | mean no. of worms per rat 16 | | | | | | | | | |
| immune | Mean | 0.45 | 0.31 | 0.16 | 0.06 | 0.004 | 0.006 | 0.00 | 0.00 | 5 |
| cells | SD | 0.08 | 0.08 | 0.03 | 0.04 | 0.010 | 0.010 | 0.00 | 0.00 | |
| Gp.E | mean no. of worms per rat 43 | | | | | | | | | |
| normal | Mean | 0.39 | 0.35 | 0.17 | 0.07 | 0.02 | 0.002 | 0.00 | 0.00 | 5 |
| cells | SD | 0.08 | 0.05 | 0.05 | 0.04 | 0.03 | 0.004 | 0.00 | 0.00 | |
| Gp.F | mean no. of worms per rat 51 | | | | | | | | | |
| day 25 | Mean | 0.46 | 0.26 | 0.13 | 0.13 | 0.01 | 0.01 | 0.00 | 0.00 | 6 |
| primary | SD | 0.08 | 0.11 | 0.10 | 0.08 | 0.02 | 0.02 | 0.00 | 0.00 | |
| infection | mean no. of worms per rat 18 | | | | | | | | | |
| Gp.A | | | | | | | | | | |
| priming | Mean | 0.39 | 0.34 | 0.16 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| controls | SD | 0.12 | 0.14 | 0.09 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.C1 | mean no. of worms per rat 59 | | | | | | | | | |
| challenge | Mean | 0.36 | 0.37 | 0.18 | 0.08 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| controls | SD | 0.14 | 0.05 | 0.11 | 0.03 | 0.01 | 0.00 | 0.00 | 0.00 | |
| Gp.C2 | mean no. of worms per rat 54 | | | | | | | | | |

Table 7:2(ii)

Expt 2, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| secondary | Mean | 0.13 | 0.09 | 0.05 | 0.23 | 0.17 | 0.10 | 0.09 | 0.15 | 5 |
| infection | SD | 0.12 | 0.13 | 0.08 | 0.21 | 0.17 | 0.14 | 0.08 | 0.11 | |
| Gp.B | mean no. of worms per rat 13 | | | | | | | | | |
| immune | Mean | 0.40 | 0.36 | 0.16 | 0.07 | 0.01 | 0.00 | 0.00 | 0.00 | 6 |
| cells | SD | 0.05 | 0.09 | 0.02 | 0.04 | 0.01 | 0.00 | 0.00 | 0.00 | |
| Gp.E | mean no. of worms per rat 61 | | | | | | | | | |
| normal | Mean | 0.38 | 0.35 | 0.16 | 0.12 | 0.01 | 0.003 | 0.00 | 0.00 | 4 |
| cells | SD | 0.06 | 0.07 | 0.04 | 0.04 | 0.02 | 0.005 | 0.00 | 0.00 | |
| Gp.F | mean no. of worms per rat 67 | | | | | | | | | |
| day 25 | Mean | 0.39 | 0.26 | 0.18 | 0.10 | 0.06 | 0.02 | 0.00 | 0.008 | 5 |
| primary | SD | 0.11 | 0.17 | 0.02 | 0.14 | 0.11 | 0.03 | 0.00 | 0.020 | |
| infection | mean no. of worms per rat 17 | | | | | | | | | |
| Gp.A | | | | | | | | | | |
| priming | Mean | 0.53 | 0.34 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| controls | SD | 0.14 | 0.01 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Gp.C1 | mean no. of worms per rat 57 | | | | | | | | | |
| challenge | Mean | 0.45 | 0.30 | 0.15 | 0.05 | 0.002 | 0.00 | 0.00 | 0.00 | 5 |
| controls | SD | 0.12 | 0.05 | 0.08 | 0.06 | 0.004 | 0.00 | 0.00 | 0.00 | |
| Gp.C2 | mean no. of worms per rat 58 | | | | | | | | | |

Table 7:2(iii)

Expt 3, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| immune cells 4000L3 donors Gp.E | Mean | 0.35 | 0.30 | 0.17 | 0.12 | 0.04 | 0.01 | 0.00 | 0.00 | 7 |
| | SD | 0.10 | 0.08 | 0.07 | 0.04 | 0.05 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 44 | | | | | | | | |
| immune cells 1000L3 donors Gp.D | Mean | 0.36 | 0.34 | 0.18 | 0.09 | 0.03 | 0.006 | 0.00 | 0.00 | 6 |
| | SD | 0.13 | 0.13 | 0.08 | 0.08 | 0.03 | 0.020 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 41 | | | | | | | | |
| day 25 primary infection 1000L3 Gp.A | Mean | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 3 | | | | | | | | |
| day 25 primary infection 4000L3 Gp.B | Mean | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 2 | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.44 | 0.35 | 0.11 | 0.09 | 0.02 | 0.004 | 0.00 | 0.00 | 5 |
| | SD | 0.14 | 0.06 | 0.04 | 0.06 | 0.03 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 60 | | | | | | | | |
| challenge controls Gp.C2 | Mean | 0.33 | 0.32 | 0.16 | 0.12 | 0.05 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.14 | 0.09 | 0.09 | 0.06 | 0.06 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 49 | | | | | | | | |

Table 7:2(iv)

Expt 4, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| immune cells 2000L3 donors Gp.E | Mean | 0.00 | 0.13 | 0.07 | 0.17 | 0.40 | 0.17 | 0.07 | 0.00 | 3 |
| | SD | 0.00 | 0.23 | 0.12 | 0.29 | 0.53 | 0.29 | 0.12 | 0.00 | |
| | mean no. of worms per rat | 3 | | | | | | | | |
| immune cells <100L3 donors Gp.D | Mean | 0.43 | 0.22 | 0.08 | 0.10 | 0.12 | 0.06 | 0.00 | 0.00 | 5 |
| | SD | 0.23 | 0.12 | 0.09 | 0.14 | 0.14 | 0.10 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 13 | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.40 | 0.28 | 0.16 | 0.13 | 0.03 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.12 | 0.06 | 0.03 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 64 | | | | | | | | |
| challenge controls day 8 Gp.C2 | Mean | 0.31 | 0.34 | 0.16 | 0.10 | 0.08 | 0.01 | 0.00 | 0.00 | 4 |
| | SD | 0.11 | 0.06 | 0.03 | 0.06 | 0.04 | 0.03 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 55 | | | | | | | | |
| challenge controls day 16 Gp.C3 | Mean | 0.50 | 0.24 | 0.12 | 0.09 | 0.02 | 0.008 | 0.00 | 0.00 | 5 |
| | SD | 0.17 | 0.12 | 0.07 | 0.06 | 0.05 | 0.020 | 0.00 | 0.00 | |
| | mean no. of worms per rat | 28 | | | | | | | | |

Table 7:2(v)

Expt 5, homogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| immune cells 2000L3 donors Gp.E | Mean | 0.19 | 0.12 | 0.11 | 0.10 | 0.22 | 0.10 | 0.06 | 0.10 | 6 |
| | SD | 0.18 | 0.14 | 0.10 | 0.09 | 0.39 | 0.11 | 0.13 | 0.08 | |
| | mean no. of worms per rat 12 | | | | | | | | | |
| immune cells <100L3 donors Gp.D | Mean | 0.45 | 0.26 | 0.02 | 0.05 | 0.02 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.06 | 0.03 | 0.08 | 0.04 | 0.02 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 29 | | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.42 | 0.27 | 0.21 | 0.09 | 0.02 | 0.07 | 0.00 | 0.00 | 3 |
| | SD | 0.16 | 0.12 | 0.02 | 0.04 | 0.02 | 0.12 | 0.00 | 0.00 | |
| | mean no. of worms per rat 53 | | | | | | | | | |
| challenge controls day 8 Gp.C2 | Mean | 0.32 | 0.30 | 0.19 | 0.09 | 0.09 | 0.02 | 0.00 | 0.00 | 4 |
| | SD | 0.09 | 0.02 | 0.04 | 0.01 | 0.04 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 57 | | | | | | | | | |
| challenge controls day 16 Gp.C2 | Mean | 0.38 | 0.29 | 0.17 | 0.13 | 0.05 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.09 | 0.05 | 0.06 | 0.05 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 43 | | | | | | | | | |

Table 7:2(vi)

Expt 6, heterogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| immune cells 2000L3 donors Gp.E | Mean | 0.26 | 0.27 | 0.24 | 0.11 | 0.03 | 0.04 | 0.04 | 0.00 | 6 |
| | SD | 0.21 | 0.15 | 0.10 | 0.10 | 0.04 | 0.07 | 0.05 | 0.00 | |
| | mean no. of worms per rat 12 | | | | | | | | | |
| immune cells <100L3 donors Gp.D | Mean | 0.31 | 0.38 | 0.13 | 0.08 | 0.11 | 0.01 | 0.00 | 0.03 | 4 |
| | SD | 0.14 | 0.10 | 0.10 | 0.06 | 0.09 | 0.02 | 0.00 | 0.07 | |
| | mean no. of worms per rat 17 | | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.30 | 0.38 | 0.18 | 0.11 | 0.02 | 0.01 | 0.00 | 0.00 | 3 |
| | SD | 0.08 | 0.03 | 0.08 | 0.05 | 0.02 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat 47 | | | | | | | | | |
| challenge controls day 8 Gp.C2 | Mean | 0.36 | 0.33 | 0.16 | 0.11 | 0.04 | 0.02 | 0.00 | 0.00 | 4 |
| | SD | 0.08 | 0.03 | 0.06 | 0.05 | 0.02 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 51 | | | | | | | | | |
| challenge controls day 16 Gp.C3 | Mean | 0.29 | 0.30 | 0.25 | 0.15 | 0.03 | 0.02 | 0.00 | 0.00 | 5 |
| | SD | 0.07 | 0.14 | 0.04 | 0.06 | 0.03 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 33 | | | | | | | | | |

Table 7:2(vii)

Expt 7, heterogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| immune cells 2000L3 donors Gp.E | Mean | 0.33 | 0.32 | 0.16 | 0.13 | 0.01 | 0.04 | 0.00 | 0.00 | 7 |
| | SD | 0.25 | 0.24 | 0.22 | 0.14 | 0.02 | 0.09 | 0.00 | 0.00 | |
| | mean no. of worms per rat 8 | | | | | | | | | |
| immune cells <100L3 donors Gp.D | Mean | 0.40 | 0.27 | 0.19 | 0.06 | 0.03 | 0.00 | 0.05 | 0.01 | 4 |
| | SD | 0.43 | 0.27 | 0.19 | 0.12 | 0.05 | 0.00 | 0.06 | 0.02 | |
| | mean no. of worms per rat 10 | | | | | | | | | |
| priming controls Gp.C1 | Mean | 0.32 | 0.32 | 0.19 | 0.10 | 0.07 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.10 | 0.07 | 0.11 | 0.05 | 0.11 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 44 | | | | | | | | | |
| challenge controls Gp.C3 | Mean | 0.38 | 0.40 | 0.16 | 0.03 | 0.02 | 0.01 | 0.00 | 0.00 | 4 |
| | SD | 0.13 | 0.27 | 0.11 | 0.04 | 0.02 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 26 | | | | | | | | | |

Table 7:2(viii)

Expt 8

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.26 | 0.19 | 0.11 | 0.11 | 0.14 | 0.02 | 0.05 | 0.14 | 6 |
| | SD | 0.26 | 0.18 | 0.10 | 0.15 | 0.21 | 0.04 | 0.06 | 0.18 | |
| | mean no. of worms per rat 16 | | | | | | | | | |
| heterologous challenge Gp.D | Mean | 0.19 | 0.17 | 0.14 | 0.21 | 0.07 | 0.07 | 0.08 | 0.09 | 4 |
| | SD | 0.12 | 0.12 | 0.07 | 0.04 | 0.06 | 0.08 | 0.10 | 0.11 | |
| | mean no. of worms per rat 15 | | | | | | | | | |
| priming homogonic S.ratti controls Gp.C1 | Mean | 0.34 | 0.39 | 0.18 | 0.11 | 0.02 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.14 | 0.04 | 0.06 | 0.08 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 62 | | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C2 | Mean | 0.25 | 0.44 | 0.22 | 0.11 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.06 | 0.09 | 0.09 | 0.06 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 51 | | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C3 | Mean | 0.26 | 0.43 | 0.15 | 0.11 | 0.04 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.12 | 0.05 | 0.12 | 0.06 | 0.02 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 29 | | | | | | | | | |

Table 7:2(ix)

Expt 9

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.27 | 0.13 | 0.15 | 0.24 | 0.07 | 0.06 | 0.04 | 0.03 | 7 |
| | SD | 0.20 | 0.16 | 0.12 | 0.22 | 0.05 | 0.06 | 0.06 | 0.06 | |
| | mean no. of worms per rat 15 | | | | | | | | | |
| heterologous challenge Gp.D | Mean | 0.17 | 0.43 | 0.14 | 0.15 | 0.09 | 0.01 | 0.00 | 0.00 | 7 |
| | SD | 0.14 | 0.28 | 0.07 | 0.15 | 0.22 | 0.03 | 0.00 | 0.00 | |
| | mean no. of worms per rat 13 | | | | | | | | | |
| priming homogonic S.ratti controls Gp.C1 | Mean | 0.35 | 0.38 | 0.16 | 0.09 | 0.02 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.03 | 0.06 | 0.06 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 71 | | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C2 | Mean | 0.38 | 0.44 | 0.12 | 0.05 | 0.005 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.19 | 0.12 | 0.02 | 0.06 | 0.01 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 36 | | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C3 | Mean | 0.32 | 0.44 | 0.16 | 0.05 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.14 | 0.08 | 0.06 | 0.07 | 0.03 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 26 | | | | | | | | | |

Table 7:2(x)

Expt 10

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.15 | 0.22 | 0.36 | 0.07 | 0.04 | 0.03 | 0.05 | 0.07 | 4 |
| | SD | 0.21 | 0.27 | 0.44 | 0.14 | 0.07 | 0.07 | 0.07 | 0.14 | |
| | mean no. of worms per rat 8 | | | | | | | | | |
| heterologous challenge Gp.D | Mean | 0.36 | 0.31 | 0.21 | 0.07 | 0.03 | 0.01 | 0.00 | 0.005 | 6 |
| | SD | 0.06 | 0.05 | 0.05 | 0.05 | 0.04 | 0.02 | 0.00 | 0.010 | |
| | mean no. of worms per rat 24 | | | | | | | | | |
| priming heterogonic S.ratti controls Gp.C1 | Mean | 0.20 | 0.41 | 0.23 | 0.10 | 0.05 | 0.01 | 0.00 | 0.00 | 3 |
| | SD | 0.07 | 0.10 | 0.06 | 0.03 | 0.04 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 58 | | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C2 | Mean | 0.32 | 0.42 | 0.10 | 0.13 | 0.03 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.10 | 0.06 | 0.05 | 0.08 | 0.04 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 33 | | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C3 | Mean | 0.37 | 0.34 | 0.17 | 0.09 | 0.03 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.09 | 0.06 | 0.07 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 53 | | | | | | | | | |

Table 7:2(xi)

Expt 11

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | Ø.42 | Ø.16 | Ø.13 | Ø.ØØ | Ø.27 | Ø.ØØ | Ø.Ø3 | Ø.ØØ | 5 |
| | SD | Ø.25 | Ø.23 | Ø.22 | Ø.ØØ | Ø.25 | Ø.ØØ | Ø.Ø6 | Ø.ØØ | |
| | mean no. of worms per rat | | | | 4 | | | | | |
| heterologous challenge Gp.D | Mean | Ø.49 | Ø.39 | Ø.Ø6 | Ø.Ø6 | Ø.ØØ | Ø.ØØ | Ø.ØØ | Ø.ØØ | 4 |
| | SD | Ø.34 | Ø.28 | Ø.Ø8 | Ø.13 | Ø.ØØ | Ø.ØØ | Ø.ØØ | Ø.ØØ | |
| | mean no. of worms per rat | | | | 8 | | | | | |
| priming heterogonic S.ratti controls Gp.C1 | Mean | Ø.33 | Ø.36 | Ø.14 | Ø.1Ø | Ø.Ø4 | Ø.Ø3 | Ø.ØØ | Ø.ØØ | 3 |
| | SD | Ø.Ø4 | Ø.14 | Ø.Ø2 | Ø.Ø7 | Ø.Ø1 | Ø.Ø4 | Ø.ØØ | Ø.ØØ | |
| | mean no. of worms per rat | | | | 47 | | | | | |
| challenge heterogonic S.ratti controls Gp.C2 | Mean | Ø.46 | Ø.31 | Ø.11 | Ø.15 | Ø.Ø2 | Ø.ØØ | Ø.ØØ | Ø.ØØ | 4 |
| | SD | Ø.18 | Ø.1Ø | Ø.11 | Ø.1Ø | Ø.Ø2 | Ø.ØØ | Ø.ØØ | Ø.ØØ | |
| | mean no. of worms per rat | | | | 29 | | | | | |
| challenge homogonic S.ratti controls Gp.C3 | Mean | Ø.46 | Ø.31 | Ø.13 | Ø.Ø6 | Ø.Ø4 | Ø.ØØ | Ø.ØØ | Ø.ØØ | 4 |
| | SD | Ø.14 | Ø.Ø9 | Ø.Ø7 | Ø.Ø3 | Ø.Ø6 | Ø.ØØ | Ø.ØØ | Ø.ØØ | |
| | mean no. of worms per rat | | | | 24 | | | | | |

Table 7:2(xii)

Expt 12

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.51 | 0.41 | 0.07 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.24 | 0.20 | 0.08 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 11 | | | | | | | | | |
| heterologous challenge homogonic S.ratti Gp.D | Mean | 0.39 | 0.27 | 0.15 | 0.13 | 0.05 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.08 | 0.17 | 0.16 | 0.08 | 0.06 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat 29 | | | | | | | | | |
| heterologous challenge heterogonic S.ratti Gp.E | Mean | 0.20 | 0.39 | 0.13 | 0.20 | 0.07 | 0.03 | 0.00 | 0.00 | 5 |
| | SD | 0.07 | 0.10 | 0.05 | 0.09 | 0.08 | 0.06 | 0.00 | 0.00 | |
| | mean no. of worms per rat 17 | | | | | | | | | |
| priming S.venezuelensis controls Gp.C1 | Mean | 0.74 | 0.24 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.10 | 0.06 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 25 | | | | | | | | | |
| challenge S.venezuelensis controls Gp.C2 | Mean | 0.65 | 0.27 | 0.06 | 0.005 | 0.01 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.31 | 0.16 | 0.13 | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 26 | | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C3 | Mean | 0.39 | 0.34 | 0.17 | 0.07 | 0.03 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.11 | 0.12 | 0.06 | 0.04 | 0.04 | 0.01 | 0.00 | 0.00 | |
| | mean no. of worms per rat 38 | | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C4 | Mean | 0.40 | 0.24 | 0.22 | 0.12 | 0.01 | 0.01 | 0.005 | 0.00 | 5 |
| | SD | 0.24 | 0.04 | 0.12 | 0.07 | 0.02 | 0.01 | 0.010 | 0.00 | |
| | mean no. of worms per rat 33 | | | | | | | | | |

Table 7:3(xiii)

Expt 13

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| homologous challenge Gp.B | Mean | 0.78 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4 |
| | SD | 0.15 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 14 | | | | | | | | | |
| heterologous homogonic S.ratti challenge Gp.D | Mean | 0.45 | 0.28 | 0.18 | 0.07 | 0.02 | 0.006 | 0.00 | 0.00 | 5 |
| | SD | 0.10 | 0.05 | 0.11 | 0.05 | 0.03 | 0.010 | 0.00 | 0.00 | |
| | mean no. of worms per rat 33 | | | | | | | | | |
| heterolgous heterogonic S.ratti challenge Gp.E | Mean | 0.45 | 0.24 | 0.16 | 0.10 | 0.03 | 0.02 | 0.00 | 0.00 | 4 |
| | SD | 0.15 | 0.15 | 0.11 | 0.05 | 0.03 | 0.04 | 0.00 | 0.00 | |
| | mean no. of worms per rat 31 | | | | | | | | | |
| priming S.venezuelensis controls Gp.C1 | Mean | 0.66 | 0.23 | 0.09 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.07 | 0.02 | 0.07 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 33 | | | | | | | | | |
| challenge S.venezuelensis controls Gp.C2 | Mean | 0.85 | 0.14 | 0.00 | 0.004 | 0.00 | 0.00 | 0.00 | 0.00 | 5 |
| | SD | 0.06 | 0.06 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 31 | | | | | | | | | |
| challenge homogonic S.ratti controls Gp.C3 | Mean | 0.34 | 0.44 | 0.16 | 0.04 | 0.01 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.07 | 0.09 | 0.05 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 46 | | | | | | | | | |
| challenge heterogonic S.ratti controls Gp.C4 | Mean | 0.49 | 0.31 | 0.12 | 0.03 | 0.03 | 0.01 | 0.00 | 0.00 | 5 |
| | SD | 0.09 | 0.08 | 0.04 | 0.03 | 0.03 | 0.02 | 0.00 | 0.00 | |
| | mean no. of worms per rat 46 | | | | | | | | | |

7:3 Resistance quotients for Expts 8-13

Table 7:3(i)

Worm data, Expts 8-13

| Expt | Treatment | Resistance quotient "$ReQ_e$" | n | SD |
|---|---|---|---|---|
| 8 | homologous challenge | 0.691 | 6 | 0.18 |
| | heterologous challenge | 0.499 | 6 | 0.19 |
| 9 | homologous challenge | 0.598 | 7 | 0.14 |
| | heterologous challenge | 0.512 | 7 | 0.26 |
| 10 | homologous challenge | 0.772 | 4 | 0.13 |
| | heterologous challenge | 0.545 | 6 | 0.13 |
| 11 | homologous challenge | 0.837 | 5 | 0.09 |
| | heterologous challenge | 0.708 | 5 | 0.15 |
| 12 | homologous challenge | 0.592 | 5 | 0.19 |
| | heterologous challenge homogonic S.ratti | 0.231 | 5 | 0.28 |
| | heterologous challenge heterogonic S.ratti | 0.480 | 5 | 0.14 |
| 13 | homologous challenge | 0.552 | 4 | 0.14 |
| | heterologous challenge homogonic S.ratti | 0.291 | 5 | 0.21 |
| | heterologous challenge heterogonic S.ratti | 0.426 | 4 | 0.12 |



Table 7:3(ii)

Egg data, Expts 8-13

| Expt | Treatment | Resistance quotient: based on no.eggs/worm ReQe | n | SD | based on no. eggs/worm/rat ReQe | n | SD |
|---|---|---|---|---|---|---|---|
| 8 | homologous challenge | 0.395 | 52 | 0.32 | 0.512 | 6 | 0.31 |
| | heterologous challenge | 0.523 | 45 | 0.34 | 0.499 | 6 | 0.12 |
| 9 | homologous challenge | 0.358 | 59 | 0.27 | 0.389 | 8 | 0.61 |
| | heterologous challenge | 0.424 | 49 | 0.23 | 0.404 | 7 | 0.15 |
| 10 | homologous challenge | 0.570 | 22 | 0.27 | 0.416 | 4 | 0.32 |
| | heterologous challenge | 0.285 | 96 | 0.29 | 0.300 | 6 | 0.11 |
| 11 | homologous challenge | 0.556 | 14 | 0.20 | 0.571 | 5 | 0.13 |
| | heterologous challenge | 0.314 | 30 | 0.27 | 0.296 | 4 | 0.16 |
| 12 | homologous challenge | 0.354 | 24 | 0.25 | 0.292 | 4 | 0.18 |
| | heterologous homogonic S.ratti challenge | 0.007 | 81 | 0.23 | 0.021 | 5 | 0.11 |
| | heterologous heterogonic S.ratti challenge | 0.198 | 45 | 0.33 | 0.183 | 5 | 0.15 |
| 13 | homologous challenge | 0.396 | 22 | 0.20 | 0.420 | 4 | 0.14 |
| | heterologous homogonic S.ratti challenge | 0.087 | 84 | 0.21 | 0.099 | 5 | 0.05 |
| | heterologous heterogonic S.ratti challenge | 0.194 | 39 | 0.22 | 0.190 | 4 | 0.04 |

8:1 Number of cpm per treatment for Expts 1-9

Table 8:1(i)

Expt 1, day 8, heterogonic S.ratti priming cells

| Rat no. | | Unstimulated control cpm | concanavalin A | | heterogonic S.ratti excretory/secretory antigen | |
|---|---|---|---|---|---|---|
| | | | cpm | differential count | cpm | differential count |
| Uninfected | | | | | | |
| A | Mean | 307 | 7310 | 7003 | 120 | -187 |
| | SD | 216 | 3100 | | 110 | |
| | n | 7 | 6 | | 5 | |
| B | Mean | 371 | 4138 | 3767 | 320 | -51 |
| | SD | 178 | 5204 | | 189 | |
| | n | 6 | 6 | | 6 | |
| Infected | | | | | | |
| 12 | Mean | 1167 | 12989 | 11822 | 792 | -375 |
| | SD | 889 | 1701 | | 340 | |
| | n | 6 | 6 | | 5 | |
| 1 | Mean | 514 | 9835 | 9321 | 711 | 197 |
| | SD | 232 | 5905 | | 448 | |
| | n | 6 | 6 | | 5 | |
| 4 | Mean | 727 | 10310 | 9583 | 2010 | 1283 |
| | SD | 83 | 5229 | | 1723 | |
| | n | 6 | 6 | | 5 | |

Table 8:1(ii)

Expt 1, day 16, heterogonic S.ratti priming cells

| Rat no. | | Unstimulated control cpm | concanavalin A | | heterogonic S.ratti excretory/secretory antigen | |
|---|---|---|---|---|---|---|
| | | | cpm | differential count | cpm | differential count |
| Uninfected | | | | | | |
| C | Mean | 107 | 1443 | 1336 | 57 | -50 |
| | SD | 101 | 230 | | 27 | |
| | n | 6 | 6 | | 6 | |
| D | Mean | 99 | 1008 | 909 | 24 | -75 |
| | SD | 80 | 222 | | 6 | |
| | n | 6 | 6 | | 6 | |
| Infected | | | | | | |
| 2 | Mean | 1138 | 2034 | 869 | 1059 | -79 |
| | SD | 322 | 640 | | 363 | |
| | n | 6 | 6 | | 6 | |
| 5 | Mean | 1804 | 3696 | 1892 | 1161 | -643 |
| | SD | 478 | 393 | | 228 | |
| | n | 6 | 6 | | 6 | |
| 11 | Mean | 170 | 1904 | 1734 | 2283 | 2113 |
| | SD | 101 | 274 | | 760 | |
| | n | 6 | 6 | | 6 | |

Table 8:1(iii)

Expt 1, day 28, heterogonic S.ratti priming cells

| Rat no. | | Unstimulated control | concanavalin A | | heterogonic S.ratti excretory/secretory antigen 1x | | 0.5x | | 0.25x | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | cpm | cpm | diff. count | cpm | diff. count | cpm | diff. count | cpm | diff. count |
| Uninfected | | | | | | | | | | |
| E | Mean | 265 | 19808 | 19543 | 126 | -139 | 87 | -178 | 94 | -171 |
| | SD | 322 | 3257 | | 75 | | 28 | | 65 | |
| | n | 5 | 5 | | 5 | | 5 | | 5 | |
| F | Mean | 82 | 23990 | 23908 | 117 | 35 | 75 | -7 | 68 | -14 |
| | SD | 38 | 9483 | | 87 | | 44 | | 51 | |
| | n | 5 | 5 | | 5 | | 5 | | 5 | |
| Infected | | | | | | | | | | |
| 9 | Mean | 167 | 10943 | 10776 | 392 | 225 | 262 | 95 | 194 | 27 |
| | SD | 60 | 1203 | | 210 | | 148 | | 87 | |
| | n | 5 | 6 | | 6 | | 6 | | 6 | |
| 6 | Mean | 237 | 8017 | 7780 | 894 | 657 | 129 | -108 | 97 | -140 |
| | SD | 125 | 616 | | 692 | | 60 | | 55 | |
| | n | 6 | 5 | | 6 | | 6 | | 6 | |
| 8 | Mean | 546 | 7398 | 6852 | 1564 | 1018 | 1320 | 744 | 625 | 79 |
| | SD | 230 | 1044 | | 666 | | 809 | | 220 | |
| | n | 6 | 5 | | 6 | | 6 | | 6 | |

* Note

diff. count = differential count

Table 8:1(iv)

Expt 2, day 28, heterogonic S.ratti priming cells

| Rat no. | | Unstimulated control | Adult excretory/secretory antigen | | | concanavalin A in ug/ml | | |
|---|---|---|---|---|---|---|---|---|
| | | cpm | homo | het | S.ven | 2.5 | 5 | 10 |
| Uninfected | | | | | | | | |
| A | Mean | 244 | 183 | 198 | 139 | 13314 | 23927 | 12721 |
| | SD | 116 | 69 | 119 | 61 | 4751 | 5140 | 6254 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -61 | -46 | -105 | 13070 | 23683 | 12477 |
| B | Mean | 1077 | 1364 | 1259 | 1439 | 45711 | 50220 | 55993 |
| | SD | 989 | 1149 | 1197 | 1437 | 11360 | 13534 | 8412 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 287 | 182 | 362 | 44634 | 49143 | 54916 |
| Infected | | | | | | | | |
| 1 | Mean | 132 | 138 | 134 | 123 | 6110 | 9142 | 4975 |
| | SD | 100 | 120 | 127 | 96 | 2376 | 5205 | 2974 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 6 | 2 | -9 | 5978 | 9010 | 4843 |
| 2 | Mean | 53 | 68 | 91 | 114 | 6406 | 6805 | 5943 |
| | SD | 15 | 20 | 26 | 105 | 1674 | 2410 | 3954 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 15 | 38 | 61 | 6353 | 6752 | 5890 |
| 3 | Mean | 44 | 65 | 84 | 334 | 1185 | 691 | 641 |
| | SD | 15 | 26 | 56 | 453 | 507 | 416 | 462 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 21 | 40 | 290 | 1141 | 647 | 597 |

* Note

S.ven = S.venezuelensis
het = heterogonic S.ratti
homo = homogonic S.ratti
Diff. Count = differential count

Table 8:1(v)

Expt 3, day 8, S.venezulensis priming cells

| Rat no. | | Unstimulated control cpm | Con A cpm | S.venezuelensis adult antigen excretory/secretory | | | homogenate | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1x | 0.5x | 0.25x | 1x | 0.5x | 0.25x |
| **Uninfected** | | | | | | | | | |
| A | Mean | 37 | 34 | 40 | 56 | 44 | 45 | 59 | 56 |
| | SD | 29 | 38 | 29 | 16 | 13 | 6 | 17 | 22 |
| | n | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -3 | 3 | 19 | 7 | 8 | 21 | 19 |
| B | Mean | 28 | 15 | 203 | 179 | 138 | 135 | 174 | 165 |
| | SD | 18 | 4 | 81 | 66 | 56 | 76 | 80 | 132 |
| | n | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -13 | 175 | 151 | 110 | 107 | 146 | 137 |
| **Infected** | | | | | | | | | |
| 7 | Mean | 86 | 30 | 82 | 97 | 218 | 279 | 261 | 205 |
| | SD | 149 | 27 | 32 | 45 | 77 | 163 | 107 | 149 |
| | n | 5 | 5 | 6 | 5 | 5 | 6 | 6 | 6 |
| | Diff. Count | | -56 | -4 | 11 | 132 | 193 | 175 | 119 |
| 1 | Mean | 18 | 20 | 90 | 102 | 91 | 115 | 128 | 112 |
| | SD | 3 | 8 | 12 | 25 | 51 | 22 | 116 | 25 |
| | n | 5 | 4 | 5 | 4 | 5 | 5 | 5 | 5 |
| | Diff. Count | | 2 | 72 | 84 | 73 | 97 | 110 | 94 |
| 6 | Mean | 27 | 16 | 74 | 257 | 134 | 184 | 180 | 206 |
| | SD | 6 | 3 | 38 | 262 | 65 | 120 | 94 | 162 |
| | n | 6 | 5 | 6 | 5 | 6 | 6 | 5 | 6 |
| | Diff. Count | | -11 | 47 | 230 | 107 | 157 | 153 | 179 |

* Note

Con A = concanavalin A

Diff. Count = differential count

Table 8:1(vi)

Expt 3, day 12, S.venezulensis priming cells

| Rat no. | | Unstimulated control cpm | Con A cpm | S.venezulensis adult antigen excretory/secretory | | | homogenate | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1x | 0.5x | 0.25x | 1x | 0.5x | 0.25x |
| **Uninfected** | | | | | | | | | |
| C | Mean | 26 | 150 | 129 | 144 | 79 | 136 | 90 | 134 |
| | SD | 14 | 59 | 41 | 77 | 22 | 48 | 29 | 60 |
| | n | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 5 |
| | Diff. Count | | 124 | 103 | 118 | 53 | 110 | 64 | 108 |
| D | Mean | 45 | 18 | 110 | 137 | 92 | 136 | 88 | 191 |
| | SD | 61 | 8 | 50 | 57 | 27 | 63 | 33 | 101 |
| | n | 6 | 5 | 5 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -27 | 65 | 92 | 47 | 91 | 43 | 146 |
| **Infected** | | | | | | | | | |
| 12 | Mean | 33 | 20 | 696 | 851 | 592 | 516 | 551 | 571 |
| | SD | 23 | 4 | 144 | 527 | 341 | 367 | 274 | 144 |
| | n | 6 | 5 | 6 | 6 | 5 | 6 | 6 | 6 |
| | Diff. Count | | -13 | 663 | 818 | 559 | 483 | 518 | 538 |
| 5 | Mean | 24 | 61 | 1165 | 1284 | 843 | 1318 | 916 | 1005 |
| | SD | 11 | 95 | 338 | 498 | 279 | 642 | 450 | 331 |
| | n | 6 | 5 | 6 | 6 | 5 | 6 | 6 | 6 |
| | Diff. Count | | 37 | 1141 | 1260 | 819 | 1294 | 892 | 981 |
| 8 | Mean | 48 | 47 | 1606 | 1294 | 610 | 1495 | 1517 | 789 |
| | SD | 59 | 48 | 803 | 598 | 133 | 451 | 306 | 279 |
| | n | 5 | 4 | 5 | 6 | 6 | 5 | 6 | 5 |
| | Diff. Count | | -1 | 1558 | 1248 | 562 | 1447 | 1469. | 741 |

* Note

Con A = concanavalin A

Diff. Count = differential count

Table 8:1(vi)

Expt 3, day 21, S.venezuelensis priming cells

| Rat no. | | Unstimulated control | Con A 2.5µg/ml sample | | | S.venezuelensis adult antigen | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | excretory/secretory | | | homogenate | | |
| | | cpm | A | B | C | 1x | 0.5x | 0.25x | 1x | 0.5x | 0.25x |
| **Uninfected** | | | | | | | | | | | |
| E | Mean | 95 | 29079 | 29239 | 243 | 81 | 80 | 84 | 53 | 83 | 102 |
| | SD | 53 | 4148 | 3847 | 314 | 37 | 23 | 33 | 12 | 30 | 65 |
| | n | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 28984 | 29144 | 148 | -14 | -15 | -11 | -42 | -12 | 7 |
| F | Mean | 85 | 23200 | 26358 | 205 | 95 | 155 | 116 | 109 | 154 | 152 |
| | SD | 21 | 2325 | 2347 | 113 | 38 | 72 | 43 | 47 | 167 | 114 |
| | n | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 23115 | 26273 | 120 | 10 | 70 | 31 | 24 | 69 | 67 |
| **Infected** | | | | | | | | | | | |
| 11 | Mean | 191 | 8675 | 12239 | 494 | 504 | 348 | 246 | 430 | 415 | 467 |
| | SD | 100 | 5758 | 412 | 244 | 209 | 44 | 151 | 207 | 143 | 234 |
| | n | 6 | 6 | 5 | 6 | 5 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 8484 | 12048 | 303 | 313 | 157 | 55 | 239 | 224 | 276 |
| 2 | Mean | 135 | 17180 | 17098 | 831 | 698 | 749 | 625 | 492 | 502 | 692 |
| | SD | 108 | 2730 | 2775 | 711 | 344 | 171 | 234 | 203 | 169 | 216 |
| | n | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 17045 | 16963 | 726 | 563 | 614 | 490 | 357 | 367 | 557 |
| 4 | Mean | 180 | 12174 | 10438 | 1089 | 971 | 856 | 925 | 1134 | 865 | 592 |
| | SD | 85 | 1298 | 681 | 750 | 371 | 507 | 268 | 542 | 307 | 220 |
| | n | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 11994 | 10258 | 839 | 791 | 676 | 745 | 954 | 685 | 412 |

* Note

Con A = concanavalin A
Diff. Count = differential count

Table 8:1(vii)

Expt 4, day 12, homogonic S.ratti priming cells

| Rat no. | | Unstimulated control | Con A 2.5µg/ml Sample | | Adult excretory/secretory antigens | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | homo | | het | | S.ven | |
| | | cpm | Ca | Cb | 0.5x | 1x | 0.5x | 1x | 0.5x | 1x |
| **Uninfected** | | | | | | | | | | |
| A | Mean | 83 | 16574 | 15388 | 68 | 85 | 70 | 77 | 90 | 107 |
| | SD | 37 | 2060 | 2118 | 25 | 21 | 36 | 51 | 81 | 107 |
| | n | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 16491 | 15305 | -15 | 2 | -13 | -6 | 7 | 24 |
| B | Mean | 90 | 25265 | 25965 | 59 | 108 | 106 | 88 | 77 | 108 |
| | SD | 32 | 3596 | 3358 | 30 | 34 | 40 | 18 | 32 | 33 |
| | n | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 25175 | 25875 | -31 | 18 | 16 | -2 | -13 | 18 |
| **Infected** | | | | | | | | | | |
| 2 | Mean | 471 | 5859 | 7119 | 411 | 439 | 1019 | 1796 | 980 | 1170 |
| | SD | 254 | 1207 | 643 | 118 | 269 | 769 | 651 | 553 | 529 |
| | n | 5 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 5388 | 6648 | -60 | -32 | 548 | 1325 | 509 | 699 |
| 3 | Mean | 152 | 5991 | 8064 | 197 | 325 | 356 | 789 | 397 | 484 |
| | SD | 30 | 2305 | 1419 | 108 | 211 | 96 | 577 | 197 | 297 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 5839 | 7912 | 45 | 173 | 204 | 637 | 245 | 3322 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
Con A = concanavalin A
Diff. Count = differential count
Ca = solution made up fresh from powder
Cb = solution stored at -20°C at a concentration of 2mg/ml before use

Table 8:1(viii)

Expt 5, day 12, heterogonic S.ratti priming cells

| Rat no. | | Unstim. control cpm | Con A cpm | Adult antigen excretory/secretory homo 0.5x | homo 1x | het 0.5x | het 1x | S.ven 0.5x | S.ven 1x | homogenate het Ha | homogenate het Hb | X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Uninfected | | | | | | | | | | | | |
| A | Mean | 234 | 8449 | 317 | 204 | 247 | 281 | 572 | 190 | 623 | 374 | 9179 |
| | SD | 133 | 1562 | 165 | 120 | 181 | 192 | 571 | 92 | 441 | 224 | 2743 |
| | n | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 5 |
| Diff. Count | | | 8215 | 83 | -30 | 13 | 47 | 338 | -44 | 389 | 140 | 8945 |
| B | Mean | 696 | 5167 | 1150 | 965 | 1479 | 783 | 878 | 947 | 744 | 826 | 3545 |
| | SD | 463 | 962 | 856 | 676 | 810 | 318 | 300 | 591 | 272 | 491 | 1040 |
| | n | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | 4471 | 454 | 269 | 783 | 87 | 182 | 251 | 48 | 130 | 2849 |
| Infected | | | | | | | | | | | | |
| 2 | Mean | 6110 | 7789 | 7508 | 16420 | 11024 | 11750 | 8136 | 9836 | 10704 | 11465 | 4460 |
| | SD | 3350 | 795 | 2159 | 3143 | 5756 | 2393 | 1916 | 2422 | 2798 | 2798 | 1455 |
| | n | 6 | 5 | 6 | 6 | 6 | 5 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | 1679 | 1398 | 10310 | 4914 | 5640 | 2026 | 3726 | 4594 | 5355 | -1650 |
| 1 | Mean | 4910 | 7231 | 10901 | 16658 | 9037 | 8862 | 8805 | 10298 | 9478 | 9984 | 7678 |
| | SD | 2502 | 666 | 1694 | 9967 | 2301 | 2493 | 2468 | 2882 | 3870 | 2593 | 4971 |
| | n | 5 | 5 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | 2411 | 5991 | 11748 | 4127 | 3952 | 3895 | 5388 | 4568 | 5078 | 2768 |
| 3 | Mean | 3684 | 13699 | 4971 | 5689 | 4031 | 5682 | 4745 | 3983 | 9639 | 5898 | 10266 |
| | SD | 1284 | 4660 | 1515 | 1460 | 1017 | 2402 | 2237 | 1266 | 2792 | 2122 | 4038 |
| | n | 5 | 5 | 6 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | 10015 | 1287 | 2005 | 347 | 1998 | 1061 | 299 | 5955 | 2214 | 6582 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
Ha = adult heterogonic S.ratti homogenate antigen stored at +4°C before use
Hb = adult heterogonic S.ratti homogenate antigen stored at -20°C before use
X = combination of mitogen and heterogonic S.ratti excretory/secretory antigen added to cells
Con A = concanavalin A
Diff. Count = differential count
Unstim. = unstimulated

Table 8:1(ix)

Expt 6, day 12, S.venezuelensis priming cells

| Rat no. | | Unstim. control cpm | Con A cpm | Adult antigen excretory/secretory homo 0.5x | homo 1x | het 0.5x | het 1x | S.ven 0.5x | S.ven 1x | Y | homogenate 1x | X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Uninfected | | | | | | | | | | | | |
| A | Mean | 271 | 2667 | 325 | 228 | 193 | 334 | 353 | 178 | 479 | 3150 | 1593 |
| | SD | 119 | 763 | 255 | 176 | 95 | 240 | 195 | 64 | 280 | 967 | 289 |
| | n | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 6 |
| Diff. Count | | | 2396 | 54 | -43 | -78 | 63 | 82 | -93 | 208 | 2879 | 1322 |
| B | Mean | 136 | 5216 | 154 | 122 | 98 | 349 | 276 | 145 | 415 | 4795 | 4415 |
| | SD | 59 | 1242 | 101 | 63 | 16 | 356 | 242 | 84 | 340 | 909 | 1532 |
| | n | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | 5080 | 18 | -12 | -38 | 213 | 140 | 9 | 279 | 4659 | 4279 |
| Infected | | | | | | | | | | | | |
| 1 | Mean | 1835 | 2962 | 775 | 1008 | 1988 | 1364 | 1533 | 2251 | 2722 | 6384 | 7223 |
| | SD | 709 | 599 | 521 | 616 | 1355 | 1025 | 968 | 1090 | 1073 | 1371 | 2423 |
| | n | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | 1127 | -1060 | -827 | 153 | -471 | -302 | 416 | 887 | 4549 | 5388 |
| 2 | Mean | 1642 | 1485 | 950 | 1007 | 1316 | 1012 | 1159 | 578 | 1874 | 3077 | 1260 |
| | SD | 1515 | 585 | 507 | 479 | 1303 | 710 | 581 | 429 | 1648 | 1429 | 1079 |
| | n | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Diff. Count | | | -157 | -692 | -635 | -326 | -630 | -483 | -1064 | 232 | 1435 | -382 |
| 3 | Mean | 2547 | 9933 | 3118 | 2823 | 2145 | 1663 | 3028 | 1748 | 2843 | 7356 | 10241 |
| | SD | 1363 | 923 | 1724 | 1310 | 1528 | 826 | 2899 | 554 | 1368 | 1245 | 1585 |
| | n | 5 | 5 | 5 | 4 | 6 | 5 | 6 | 6 | 6 | 6 | * 6 |
| Diff. Count | | | 7386 | 571 | 276 | -402 | -884 | 481 | -799 | 296 | 4809 | 7694 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
X = heterogonic S.ratti homogenate antigen and mitogen added to cells
Y = heterogonic S.ratti excretory/secretory antigen and mitogen added to cells
Con A = concanavalin A
unstim. = unstimulated
Diff. Count = differential count

Table 8:1(xi)

Expt 7, day 12, heterogonic S.ratti priming cells

| Rat no. | | Unstim. control cpm | Adult antigen 1x het | Adult antigen 1x homo | Adult antigen 1x Sven | L3 antigen 1x homo | L3 antigen 1x hat | L3 antigen 1x S.ven | Con A Sample Ca | Con A Sample Cb |
|---|---|---|---|---|---|---|---|---|---|---|
| **Uninfected** | | | | | | | | | | |
| A | Mean | 122 | 149 | 109 | 105 | 74 | 222 | 233 | 8462 | 8026 |
| | SD | 67 | 102 | 45 | 30 | 32 | 226 | 81 | 1408 | 867 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 |
| | Diff. Count | | 27 | -13 | -17 | -48 | 100 | 111 | 8340 | 7904 |
| B | Mean | 1152 | 625 | 999 | 470 | 507 | 747 | 641 | 3178 | 4548 |
| | SD | 529 | 335 | 508 | 108 | 308 | 266 | 290 | 482 | 971 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 |
| | Diff. Count | | -527 | -153 | -682 | -645 | -405 | -511 | 2018 | 3396 |
| **Infected** | | | | | | | | | | |
| 1 | Mean | 4981 | 8365 | 7668 | 8581 | 9546 | 7683 | 9905 | 5014 | 6342 |
| | SD | 1611 | 3224 | 2467 | 4910 | 1847 | 1590 | 1848 | 803 | 699 |
| | n | 5 | 5 | 6 | 5 | 6 | 6 | 6 | 6 | 5 |
| | Diff. Count | | 3384 | 2687 | 3600 | 4565 | 2702 | 4924 | 33 | 1361 |
| 2 | Mean | 8212 | 10903 | 15533 | 10610 | 14807 | 11979 | 11324 | 7585 | - |
| | SD | 5355 | 3678 | 3672 | 4936 | 6569 | 3220 | 3553 | 1252 | - |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | |
| | Diff. Count | | 2691 | 7321 | 2398 | 6595 | 3767 | 3112 | -627 | |
| 3 | Mean | 39027 | 38005 | 42488 | 41387 | 45682 | 39201 | 44240 | 8842 | 16093 |
| | SD | 18444 | 3860 | 5680 | 4832 | 5383 | 13215 | 4747 | 2635 | 14921 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 5 |
| | Diff. Count | | -1022 | 3461 | 2360 | 6655 | 174 | 5213 | -30185 | -22934 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
unstim. = unstimulated
ConA = concanavalin A
Ca = mitogen solution made up fresh from powder
Cb = mitogen soultion was stored at -20°C at a concentration of 2mg/ml before use
Diff. Count = differential count

Table 8:1(xii)

Expt 7, day 16, heterogonic S.ratti priming cells

| Rat no. | | Unstim. control cpm | Adult antigen 1x het | Adult antigen 1x homo | Adult antigen 1x Sven | L3 antigen 1x homo | L3 antigen 1x het | L3 antigen 1x S.ven | Con A sample Ca | Con A sample Cb |
|---|---|---|---|---|---|---|---|---|---|---|
| **Uninfected** | | | | | | | | | | |
| C | Mean | 5952 | 4120 | 4919 | 2918 | 3588 | 4040 | 4060 | 8564 | 8663 |
| | SD | 2149 | 2457 | 2120 | 911 | 993 | 1862 | 2295 | 1958 | 875 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 |
| | Diff. Count | | -1832 | -1033 | -3034 | -2364 | -1912 | -1892 | 2612 | 2711 |
| D | Mean | 701 | 540 | 760 | 278 | 86 | 613 | 482 | 3924 | 3842 |
| | SD | 387 | 258 | 417 | 241 | 56 | 238 | 188 | 559 | 670 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 4 |
| | Diff. Count | | -161 | 59 | -423 | -615 | -88 | -219 | 3223 | 3141 |
| **Infected** | | | | | | | | | | |
| 3 | Mean | 8179 | 10564 | 18354 | 13149 | 13345 | 22850 | 15308 | 6734 | 6672 |
| | SD | 2279 | 5132 | 2907 | 3849 | 4976 | 4782 | 4717 | 452 | 736 |
| | n | 5 | 6 | 6 | 5 | 6 | 6 | 6 | 5 | 6 |
| | Diff. Count | | 2385 | 10175 | 4970 | 5166 | 14671 | 7129 | -1445 | -1507 |
| 4 | Mean | 10069 | 10984 | 10726 | 8027 | 11573 | 16094 | 9260 | 7211 | 7166 |
| | SD | 1917 | 2268 | 3046 | 2378 | 4717 | 3955 | 2740 | 999 | 1489 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 5 |
| | Diff. Count | | 915 | 657 | -2042 | 1504 | 6025 | -809 | -2858 | -2903 |
| 6 | Mean | 14510 | 17281 | 23066 | 17224 | 19896 | 22094 | 22138 | 9267 | 8828 |
| | SD | 4173 | 4186 | 4016 | 2672 | 1764 | 2662 | 3479 | 1154 | 689 |
| | n | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 |
| | Diff. Count | | 2771 | 8556 | 2714 | 5386 | 7584 | 7628 | -5223 | -5682 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
ConA = concanavalin A
Ca = mitogen made up fresh from powder
Cb = mitogen solution had been stored at -20°C at a concentration of 2mg/ml before use
unstim. = unstimulated
Diff. Count = differential count
L3 = third-stage larval homogenate antigen

Table 8:1(xiii)

Expt 8, day 12, heterogonic S.ratti priming cells

| Rat no. | | Unstim. control cpm | Adult antigen 1x hetero | Adult antigen 1x homo | Adult antigen 1x Sven | L3 antigen 1x hetero | L3 antigen 1x homo | Con A |
|---|---|---|---|---|---|---|---|---|
| **Uninfected** | | | | | | | | |
| A | Mean | 664 | 612 | 737 | 983 | 355 | 111 | 12849 |
| | SD | 409 | 613 | 731 | 554 | 311 | 66 | 1691 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -52 | 73 | 319 | -309 | -553 | 12185 |
| B | Mean | 295 | 338 | 304 | 309 | 112 | - | 11541 |
| | SD | 180 | 164 | 242 | 253 | 45 | - | 1523 |
| | n | 6 | 6 | 6 | 6 | 6 | | 6 |
| | Diff. Count | | 43 | 9 | 14 | -183 | | 11246 |
| **Infected** | | | | | | | | |
| 1 | Mean | 4425 | 3474 | 4085 | 3941 | 3351 | 1811 | 16111 |
| | SD | 1384 | 1870 | 1439 | 1219 | 1066 | 1049 | 2689 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -951 | -340 | -484 | -1074 | -2611 | 11686 |
| 3 | Mean | 2064 | 678 | 1893 | 1023 | 1487 | 1787 | 14412 |
| | SD | 1892 | 644 | 1308 | 568 | 802 | 1420 | 1742 |
| | n | 6 | 6 | 6 | 6 | 6 | 3 | 6 |
| | Diff. Count | | -678 | -171 | -1041 | -577 | -277 | 12348 |
| 5 | Mean | 7963 | 9299 | 10080 | 8003 | 7119 | 10335 | 19469 |
| | SD | 2175 | 3247 | 3818 | 1562 | 4453 | 2181 | 3079 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 1336 | 2117 | 40 | -844 | 2372 | 11506 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
ConA = concanavalin A
unstim. = unstimulated
Diff. Count = differential count
L3 = third-stage larval homogenate antigen

Table 8:1(xiv)

Expt 8, day 16, heterogonic S.ratti priming cells

| Rat No. | | Unstim. control cpm | Adult antigen 1x hetero | Adult antigen 1x homo | Adult antigen 1x Sven | L3 antigen 1x hetero | L3 antigen 1x homo | Con A |
|---|---|---|---|---|---|---|---|---|
| **Uninfected** | | | | | | | | |
| C | Mean | 5373 | 4240 | 4097 | 3923 | 4448 | 6198 | 25531 |
| | SD | 1632 | 978 | 491 | 654 | 1660 | 1717 | 8961 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | -1133 | -1276 | -1450 | -925 | 825 | 20185 |
| D | Mean | 4005 | 3085 | 3875 | 3555 | 4045 | 5798 | 22810 |
| | SD | 1082 | 683 | 961 | 926 | 699 | 1999 | 3897 |
| | n | 6 | 6 | 6 | 6 | 6 | | 6 |
| | Diff. Count | | -920 | -130 | -450 | 40 | 1793 | 18805 |
| E | Mean | 7366 | 8317 | 7586 | 7132 | 4433 | 8283 | 17118 |
| | SD | 1505 | 3042 | 2560 | 2077 | 1755 | 3843 | 3205 |
| | n | 6 | 6 | 6 | 6 | 4 | 4 | 6 |
| | Diff. Count | | 951 | 220 | -234 | -2933 | 917 | 9752 |
| **Infected** | | | | | | | | |
| 2 | Mean | 19428 | 20752 | 25655 | 24426 | 30625 | 35526 | 47060 |
| | SD | 4200 | 3017 | 5433 | 4052 | 3213 | 5519 | 6975 |
| | n | 6 | 6 | 6 | 6 | 6 | 3 | 6 |
| | Diff. Count | | 1324 | 6227 | 4998 | 11197 | 16098 | 27632 |
| 4 | Mean | 32815 | 37708 | 33869 | 28633 | 45341 | 47467 | 25507 |
| | SD | 1799 | 7505 | 3511 | 5015 | 9037 | 5225 | 2886 |
| | n | 6 | 6 | 6 | 6 | 6 | 3 | 6 |
| | Diff. Count | | 4893 | 1054 | -4182 | 12526 | 14652 | -7308 |
| 6 | Mean | 6887 | 11531 | 9898 | 11057 | 17213 | 18845 | 27773 |
| | SD | 2596 | 4640 | 2355 | 1884 | 1049 | 3018 | 3570 |
| | n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | Diff. Count | | 4644 | 3011 | 4170 | 10326 | 11958 | 20886 |

* Note

homo = homogonic S.ratti
het = heterogonic S.ratti
S.ven = S.venezuelensis
ConA = concanavalin A
unstim. = unstimulated
Diff. Count = differential count
L3 = third-stage larval homogenate antigen

Table 8:1(xv)

Effect of cell density on amount of lymphocyte stimulation, Expt 9

| Rat no. | | cell density A | | | B | | | C | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Unstim. control | Con A | Diff. Count | Unstim. control | Con A | Diff. Count | Unstim. control | Con A | Diff. Count |
| 1 | Mean | 262 | 4646 | 4384 | 118 | 11407 | 11289 | 140 | 14427 | 144317 |
| | SD | 171 | 1518 | | 39 | 5057 | | 55 | 2699 | |
| | n | 5 | 10 | | 5 | 10 | | 5 | 11 | |
| 2 | Mean | 155 | 5194 | 5039 | 105 | 7602 | 7497 | 229 | 8850 | 8621 |
| | SD | 66 | 2209 | | 35 | 1097 | | 162 | 1911 | |
| | n | 5 | 11 | | 5 | 11 | | 5 | 11 | |
| 3 | Mean | 142 | 9252 | 9110 | 135 | 13005 | 12869 | 244 | 10245 | 10001 |
| | SD | 42 | 2550 | | 51 | 3654 | | 68 | 1493 | |
| | n | 5 | 10 | | 5 | 11 | | 5 | 10 | |

Where:-
A = $4x10^5$ cells per well
B = $2x10^5$ cells per well
C = $1x10^5$ cells per well
Diff. count = differential count
Con.A = concanavalin A

## 8:2 Summary of worm data

Table 8:2(i)

Expt 1, heterogonic S.ratti

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 8 <100L3 | 0.302 | 3 | 0.06 | 5.333 | 3 | 0.95 | 5.357 | 14 | 1.21 |
| day 8 2000L3 | 0.330 | 3 | 0.03 | 5.183 | 3 | 0.38 | 5.183 | 60 | 1.42 |
| day 16 2000L3 | 0.165 | 3 | 0.10 | 1.883 | 3 | 0.43 | 1.883 | 60 | 1.04 |
| day 28 2000L3 | 0.000 | 3 | 0.00 | | | | | | |
| mean exact dose | 98.33 | | | | | | | | |

Table 8:2(ii)

Expt 2, heterogonic S.ratti, Day 28

| Treatment | mean proportion of the dose recovered | n | SD |
|---|---|---|---|
| 2000L3 | 0.00 | 3 | 0.00 |

Table 8:2(iii)

Expt 3, S.venezuelensis

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 8 <100L3 | 0.421 | 2 | 0.10 | 7.192 | 3 | 0.38 | 7.278 | 36 | 1.21 |
| day 8 2000L3 | 0.244 | 3 | 0.05 | 6.750 | 3 | 0.22 | 6.750 | 60 | 1.13 |
| day 12 2000L3 | 0.220 | 3 | 0.04 | 3.100 | 3 | 1.16 | 3.100 | 60 | 1.54 |
| day 21 2000L3 | 0.184 | 3 | 0.04 | 3.217 | 3 | 0.60 | 3.217 | 60 | 1.43 |
| mean exact dose | 96.00 | | | | | | | | |

Table 8:2(iv)

Expt 4, homogonic S.ratti

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 12 2000L3 | 0.301 | 3 | 0.03 | 7.250 | 3 | 0.61 | 7.250 | 60 | 1.47 |

Table 8:2(v)

Expt 5, heterogonic S.ratti

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 12 2000L3 | 0.227 | 3 | 0.01 | 4.917 | 3 | 0.20 | 4.917 | 60 | 1.23 |

Table 8:2(vi)

Expt 6, S.venezuelensis

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 12 2000L3 | 0.117 | 3 | 0.05 | 4.400 | 3 | 0.13 | 4.400 | 60 | 1.20 |

Table 8:2(vii)

Expt 7, heterogonic S.ratti

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 12 2000L3 | 0.432 | 3 | 0.11 | 3.533 | 3 | 0.81 | 3.533 | 60 | 1.48 |
| day 16 2000L3 | 0.059 | 3 | 0.02 | 2.065 | 3 | 0.20 | 2.050 | 60 | 1.27 |

Table 8:2(viii)

Expt 8, heterogonic S.ratti

| Treatment | mean prop. of the dose recovered | n | SD | mean no. of eggs in utero/worm/ rat | n | SD | mean no. of eggs in utero/worm | n | SD |
|---|---|---|---|---|---|---|---|---|---|
| day 12 2000L3 | 0.289 | 3 | 0.05 | 4.533 | 3 | 0.51 | 4.533 | 60 | 0.08 |
| day 16 2000L3 | 0.101 | 3 | 0.06 | 2.050 | 3 | 0.20 | 2.350 | 60 | 1.53 |

## 8:3 Summary of adults distribution along the intestine in Expts 1-8

Table 8:3(i)

Expt 1, heterogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 8 | Mean | 0.33 | 0.44 | 0.17 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | 3 |
| <100L3 | SD | 0.10 | 0.02 | 0.08 | 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 30 | | | | | | | | | |
| day 8 | Mean | 0.42 | 0.27 | 0.17 | 0.09 | 0.04 | 0.01 | 0.004 | 0.001 | 3 |
| 2000L3 | SD | 0.03 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.004 | 0.002 | |
| | mean no. of worms per rat 660 | | | | | | | | | |
| day 16 | Mean | 0.24 | 0.19 | 0.18 | 0.10 | 0.08 | 0.05 | 0.06 | 0.13 | 3 |
| 2000L3 | SD | 0.09 | 0.12 | 0.01 | 0.04 | 0.03 | 0.01 | 0.03 | 0.03 | |
| | mean no. of worms per rat 0 | | | | | | | | | |

Table 8:3(ii)

Expt 3, S.venezuelensis

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| day 8 | Mean | 0.76 | 0.23 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| <100L3 | SD | 0.05 | 0.06 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 40 | | | | | | | | | |
| day 8 | Mean | 0.64 | 0.34 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 3 |
| 2000L3 | SD | 0.08 | 0.07 | 0.01 | 0.001 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 488 | | | | | | | | | |
| day 12 | Mean | 0.66 | 0.29 | 0.04 | 0.01 | 0.003 | 0.00 | 0.00 | 0.00 | 3 |
| 2000L3 | SD | 0.10 | 0.08 | 0.02 | 0.01 | 0.004 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 440 | | | | | | | | | |
| day 21 | Mean | 0.61 | 0.35 | 0.03 | 0.003 | 0.001 | 0.00 | 0.00 | 0.00 | 3 |
| 2000L3 | SD | 0.02 | 0.03 | 0.02 | 0.003 | 0.003 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 368 | | | | | | | | | |

Table 8:3(iii)

Expts 4-6

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| **Expt 4, homogonic S.ratti** | | | | | | | | | | |
| day 12 2000L3 | Mean | 0.27 | 0.24 | 0.23 | 0.12 | 0.07 | 0.05 | 0.01 | 0.001 | 3 |
| | SD | 0.09 | 0.03 | 0.06 | 0.02 | 0.05 | 0.06 | 0.02 | 0.001 | |
| | mean no. of worms per rat 602 | | | | | | | | | |
| **Expt 5, heterogonic S.ratti** | | | | | | | | | | |
| day 12 2000L3 | Mean | 0.47 | 0.03 | 0.15 | 0.05 | 0.02 | 0.01 | 0.004 | 0.001 | 3 |
| | SD | 0.02 | 0.03 | 0.03 | 0.03 | 0.02 | 0.01 | 0.004 | 0.001 | |
| | mean no. of worms per rat 454 | | | | | | | | | |
| **Expt 6, S.venezuelensis** | | | | | | | | | | |
| day 12 2000L3 | Mean | 0.65 | 0.30 | 0.04 | 0.01 | 0.001 | 0.00 | 0.00 | 0.00 | 3 |
| | SD | 0.05 | 0.04 | 0.02 | 0.01 | 0.002 | 0.00 | 0.00 | 0.00 | |
| | mean no. of worms per rat 234 | | | | | | | | | |

Table 8:2(iv)

Expts 7 and 8, heterogonic S.ratti

| Treatment | | Proportion of worms in each gut section 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Rat n |
|---|---|---|---|---|---|---|---|---|---|---|
| **Expt 7** | | | | | | | | | | |
| day 12 2000L3 | Mean | 0.38 | 0.26 | 0.14 | 0.12 | 0.06 | 0.04 | 0.010 | 0.001 | 3 |
| | SD | 0.07 | 0.04 | 0.03 | 0.03 | 0.02 | 0.02 | 0.002 | 0.001 | |
| | mean no. of worms per rat 864 | | | | | | | | | |
| day 16 2000L3 | Mean | 0.26 | 0.14 | 0.07 | 0.08 | 0.08 | 0.07 | 0.06 | 0.23 | 3 |
| | SD | 0.19 | 0.05 | 0.02 | 0.07 | 0.01 | 0.05 | 0.07 | 0.12 | |
| | mean no. of worms per rat 118 | | | | | | | | | |
| **Expt 8** | | | | | | | | | | |
| day 12 2000L3 | Mean | 0.31 | 0.31 | 0.19 | 0.11 | 0.05 | 0.03 | 0.010 | 0.001 | 3 |
| | SD | 0.11 | 0.06 | 0.02 | 0.03 | 0.01 | 0.01 | 0.001 | 0.002 | |
| | mean no. of worms per rat 578 | | | | | | | | | |
| day 16 2000L3 | Mean | 0.29 | 0.28 | 0.16 | 0.10 | 0.07 | 0.02 | 0.02 | 0.06 | 3 |
| | SD | 0.20 | 0.05 | 0.02 | 0.09 | 0.07 | 0.02 | 0.02 | 0.07 | |
| | mean no. of worms per rat 202 | | | | | | | | | |

8:4 Antigens used throughout Expts 1-8

Expt 1

a) adult heterogonic S.ratti homogenate antigen pool 1

Expt 2

a) adult heterogonic S.ratti excretory/secretory antigen pool 1

b) adult homogonic S.ratti excretory/secretory antigen pool 1

c) adult S.venezuelensis excretory/secretory antigen pool 2

Expt 3

a) adult S.venezuelensis homogenate antigen pool 1

b) adult S.venezuelensis excretory/secretory antigen pool 1

Expt 4

a) adult heterogonic S.ratti excretory/secretory antigen pool 2

b) adult homogonic S.ratti excretory/secretory antigen pool 2

c) adult S.venezuelensis excretory/secretory antigen pool 2

Expt 5

a) adult heterogonic S.ratti excretory/secretory antigen pool 1

b) adult homogonic S.ratti excretory/secretory antigen pool 2

c) adult S.venezuelensis excretory/secretory antigen pool 3

d) adult heterogonic S.ratti homogenate antigen pool 2

Expt 6

a) adult heterogonic S.ratti excretory/secretory antigen pool 1

b) adult homogonic S.ratti excretory/secretory antigen pool 2

c) adult S.venezuelensis excretory/secretory antigen pool 3

d) adult heterogonic S.ratti homogenate antigen pool 2

Expt 7

a) adult heterogonic S.ratti excretory/secretory antigen pool 1

b) adult homogonic S.ratti excretory/secretory antigen pool 2

c) adult S.venezuelensis excretory/secretory antigen pool 3

d) third-stage heterogonic S.ratti homogenate antigen pool 1

e) third-stage homogonic S.ratti homogenate antigen pool 1

f) third-stage S.venezuelensis homogenate antigen pool 1

Expt 8

a) adult heterogonic S.ratti excretory/secretory antigen pool 1

b) adult homogonic S.ratti excretory/secretory antigen pool 2

c) adult S.venezuelensis excretory/secretory antigen pool 3

d) third-stage heterogonic S.ratti homogenate antigen pool 2

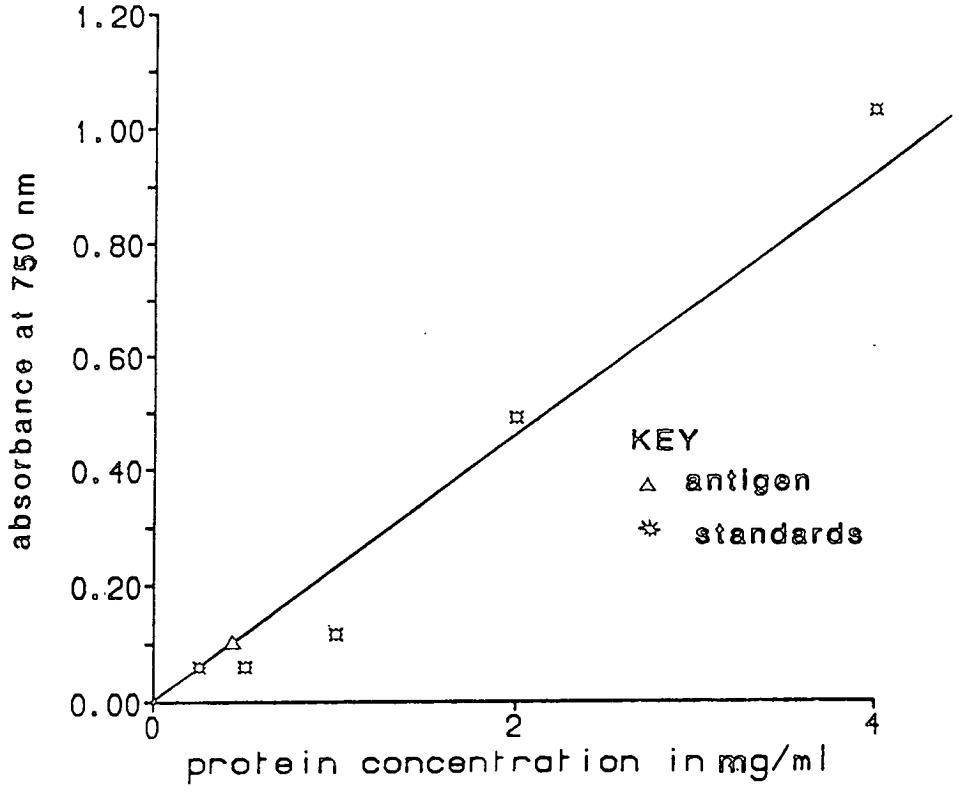
e) third-stage homogonic S.ratti homogenate antigen pool 2

8:5 Protein determination of antigens

The protein concentration of the antigens was determined using a micro-Lowry assay (Section 2:8, Chapter 2, page 43 ). In order to calculate the protein concentration of the test sample from the results obtained a standard curve, using the results of the protein standards, was plotted and the best straight line fitted by eye. The concentration of the test sample was read off this curve and unfortunately no protein was present. An example of the results obtained is shown in Fig.8:1 where the concentration of a heterogonic S.ratti antigen was determined.

Fig.8:1

Example of the results from a Lowry assay




concentration of antigen 47µg/ml

## 8:6 Statistical analysis of data

Since extensive analysis was carried out on the results of each experiment only the type of tests carried out on one experiment are included as an example, the results of other experiment were treated in the same manner.

### Experiment 8, heterogonic S.ratti priming cells

Table 8:6(i)

Two-level nested anova comparing activity of unstimulated cells from infected and uninfected rats

a) Day 12

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 1 | 38.143 | 38.143 | 12.08 |
| Among Subgps. | 3 | 9.472 | 3.157 | 10.12 |
| Within | 25 | 7.788 | 0.312 | |
| Total | 29 | 55.403 | | |

$F_{(1,3)}=10.13$ 5% $F_{(3,25)}=7.45$ 0.1%

b) Day 16

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 1 | 10.978 | 10.978 | 4.62 |
| Among Subgps. | 4 | 9.504 | 2.376 | 30.86 |
| Within | 30 | 2.304 | 0.077 | |
| Total | 35 | 22.786 | | |

$F_{(1,4)}=7.71$ 5% $F_{(4,30)}=6.12$ 0.1%

Two-level nested anova comparing response of infected and uninfected rats to concanavalin A stimulation

Table 8:6(ii)

a) Day 12

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Gps. | 1 | 0.000002 | 0.000002 | $1.25 \times 10^{-4}$ |
| Among Subgps. | 3 | 0.048 | 0.016 | 0.42 |
| Within | 25 | 0.098 | 0.038 | |
| Total | 29 | 1.007 | | |

b) Day 16

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Gps. | 1 | 254.858 | 254.858 | 0.87 |
| Among Subgps. | 4 | 1177.983 | 294.496 | 1774.07 |
| Within | 24 | 3.956 | 0.166 | |
| Total | 29 | 1436.827 | | |

$F_{(4,24)} = \underline{6.59}$ 0.1%

c) Day 16, omitting rat 4

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Gps. | 1 | 1.125 | 1.125 | 2.28 |
| Among Subgps. | 3 | 1.478 | 0.493 | 4.93 |
| Within | 20 | 1.996 | 0.100 | |
| Total | 24 | 4.599 | | |

$F_{(1,3)} = \underline{10.13}$ 5% $F_{(3,20)} = \underline{4.94}$ 1%

Table 8:6(iii)

Two-level nested anova comparing response of uninfected and infected cells to stimulation with heterogonic S.ratti excretory/secretory antigen

a) Day 12

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Gps. | 1 | 65.606 | 65.606 | 0.97 |
| Among Subgps. | 3 | 202.557 | 67.519 | 2.00 |
| Within | 25 | 841.906 | 33.676 | |
| Total | 29 | 1110.069 | | |

$F_{(3,25)}=2.99$ 5%

b) Day 16

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Gps. | 1 | 619.678 | 619.678 | 12.98 |
| Among Subgps. | 4 | 190.954 | 47.738 | 1.24 |
| Within | 30 | 1157.532 | 38.584 | |
| Total | 35 | 1968.164 | | |

$F_{(1,4)}=12.22$ 2.5% $F_{(4,30)}=2.69$ 5%

Table 8:6(iv)

Two-level nested anova comparing response of cells from uninfected and infected rats to stimulation with heterogonic S.ratti third-stage larval homogenate antigen

a) Day 12

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Gps. | 1 | 34.217 | 34.127 | 1.22 |
| Among Subgps. | 3 | 83.814 | 27.938 | 0.81 |
| Within | 25 | 867.139 | 34.686 | |
| Total | 29 | 985.170 | | |

$F_{(1,3)}=10.13$ 5%

b) Day 16

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 1 | 807.045 | 807.045 | 20.34 |
| Among Subgps. | 3 | 119.003 | 39.668 | 2.03 |
| Within | 15 | 292.675 | 19.512 | |
| Total | 19 | 1218.724 | | |

$F_{(1,3)}=17.4$ 5% $F_{(3,15)}=3.29$ 5%

Table 8:6(v)

Two-way anova comparing response of cells from infected rats to stimulation with adult excretory/secretory antigens

a) Day 12

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Cols. | 2 | 177.876 | 88.938 | 2.39 |
| Among Rows | 2 | 496.793 | 248.397 | 6.68 |
| Interaction | 4 | 63.077 | 15.769 | 0.42 |
| Among Gps. | 8 | 737.746 | 92.218 | |
| Within | 45 | 1673.763 | 37.195 | |
| Total | | 2411.509 | | |

$F_{(2,40)}=3.23$ 5% $F_{(2,40)}=5.18$ 1%

b) Day 16

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Cols. | 2 | 63.886 | 31.943 | 0.814 |
| Among Rows | 2 | 266.101 | 133.050 | 3.39 |
| Interaction | 4 | 443.838 | 110.958 | 2.83 |
| Among Gps. | 8 | 773.818 | 96.727 | |
| Within | 45 | 1766.910 | 39.265 | |
| Total | | 2540.729 | | |

$F_{(2,40)}=3.23$ 5% $F_{(4,40)}=2.61$ 5%

c) Day 16 - Single classification anova on data because of significant interaction

i) heterogonic S.ratti antigen

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 2 | 34.474 | 17.237 | 0.38 |
| Within | 15 | 684.145 | 45.610 | |
| Total | | 718.620 | | |

ii) homogonic S.ratti antigen

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 2 | 8.999 | 4.500 | 0.11 |
| Within | 15 | 637.323 | 42.488 | |
| Total | | 669.185 | | |

iii) S.venezuelensis antigen

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Gps. | 2 | 666.459 | 333.229 | 11.22 |
| Within | 15 | 445.442 | 29.696 | |
| Total | | 1111.900 | | |

$F_{(2,15)}=6.36$ 1%

Table 8:6(vi)

Two-way anova comparing response of cells from infected rats to stimulation with third-stage larval antigens

a) Day 12, rats 1 and 5 only

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Cols. | 1 | 7.560 | 7.560 | 0.22 |
| Among Rows | 1 | 508.115 | 508.116 | 14.80 |
| Interaction | 1 | 95.401 | 95.401 | 2.78 |
| Among Gps. | 3 | 611.077 | 203.692 | |
| Within | 20 | 686.628 | 34.331 | |
| Total | | 1297.705 | | |

$F_{(1,20)}=\underline{14.82}$ 0.1% $F_{(1,20)}=\underline{4.35}$ 5%

b) Day 16 - 2 rats only

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Cols. | 1 | 0.227 | 0.277 | 1.13 |
| Among Rows | 1 | 0.022 | 0.022 | 0.09 |
| Interaction | 1 | 0.056 | 0.056 | 0.023 |
| Among Gps. | 3 | 0.355 | 0.118 | |
| Within | 20 | 4.903 | 0.245 | |
| Total | | 5.258 | | |

$F_{(1,20)}=\underline{4.35}$ 5%

c) day 16 - All rats

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Among Cols. | 1 | 1.153 | 1.153 | 7.43 |
| Among Rows | 2 | 0.375 | 0.187 | 1.21 |
| Interaction | 2 | 0.614 | 0.307 | 0.72 |
| Among Gps. | 5 | 2.142 | 0.428 | |
| Within | 6 | 0.932 | | |
| Total | | 3.074 | | |

$F_{(1,6)}=\underline{5.99}$ 5% $F_{(2,6)}=\underline{5.14}$ 5%

Table 8:6(vii)

Two-level anova comparing response of cells from infected rats to stimulation with heterogonic adult and third-stage larval antigens

a) Day 12

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Cols. | 1 | 14.887 | 14.887 | 0.36 |
| Among Rows | 2 | 219.025 | 109.512 | 2.61 |
| Interaction | 2 | 45.487 | 22.744 | 0.54 |
| Among Gps. | 5 | 279.399 | 55.880 | |
| Within | 30 | 1257.161 | 41.905 | |
| Total | | 1536.599 | | |

$F_{(2,30)}=3.32$ 5%

b) Day 16 - 2 rats only

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Cols. | 1 | 73.675 | 73.675 | 3.67 |
| Among Rows | 1 | 1.193 | 1.193 | 0.06 |
| Interaction | 1 | 1.005 | 1.005 | 0.05 |
| Among Gps. | 3 | 75.872 | 25.291 | |
| Within | 20 | 401.863 | 20.093 | |
| Total | | 477.735 | | |

$F_{(1,20)}=4.35$ 5%

c) Day 16 - all rats

| Source of Variation | df | SS | MS | $F_s$ |
|---|---|---|---|---|
| Among Cols. | 1 | 40.150 | 40.528 | 0.058 |
| Among Rows | 2 | 25.056 | 12.528 | 0.954 |
| Interaction | 2 | 33.049 | 16.524 | 1.26 |
| Among Gps. | 5 | 98.255 | 19.651 | |
| Within | 6 | 78.773 | 13.129 | |
| Total | | 177.029 | | |

$F_{(1,6)}=5.99$ 5% $F_{(2,6)}=5.14$ 5%